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ON THE SCATTERING OF LIGHT BY SUPERSONIC WAVES

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1. *Introduction.*—In a paper published in 1922 Leon Brillouin[1] treated the problem of light scattering. In accordance with the fact that for low temperatures Einstein's theory of specific heat has to be abandoned for Debye's theory, Brillouin attributes the thermal density fluctuations in the body, which, in his theory, as in a previous theory of Einstein's,[2] are responsible for the scattering to a superposition of sound waves. He tries to apply his theoretical results to the explanation of x-ray scattering. We know now that this application is far from correct, as for such short waves the electronic density changes due to the atomic or molecular structure are much more important than the thermal fluctuations. For light waves, however, with a wave-length much longer than molecular distances, Brillouin's analysis leads to some remarkable results. They can be stated in the following manner. Suppose the primary light travels in figure 1 in a direction characterized by a vector $\vec{S}_0$ of length unity in this direction. Let it be assumed that of the scattered light a part is observed traveling in another direction characterized by a unit vector $\vec{S}$. Then firstly, of the sound waves of all possible directions, only those are important for the scattering which are traveling in or opposite to the direction of the vector $\vec{s} = \vec{S} - \vec{S}_0$. This can also be expressed by saying that the planes of the sound waves have to be situated such that the scattered light can be considered as optically reflected by these planes. But there is a second limitation. Of all the sound waves of direction $\pm \vec{s}$, only those of a definite wave-length Λ are effective. This wave-length is $\Lambda = \lambda/s$, if λ is the wave-length of the light and s is the length of the vector $\vec{s}$, which is $2 \sin \theta/2$, calling θ the angle between the primary and the secondary ray. This last condition can be expressed by saying that the consecutive planes of maximum density in the sound wave must be

separated by a distance Λ such that the well-known relation of Bragg holds. In this case the light rays reflected by the consecutive planes will have path differences of one wave-length (of light) each and therefore the reflections will be strong. So we are left with only two sound waves, traveling with the velocity of sound q, one in the direction $+\vec{s}$ and the other in the direction $-\vec{s}$. The frequency of the reflected light, according to Doppler's principle, will be changed, and instead of the primary frequency ν, we should find in the scattered light the two frequencies:

$$\nu = \nu_0 [1 \pm 2n\, q/c \sin \theta/2]$$

where c is the velocity of light in vacuo and n is the index of refraction of the medium.

Gross[3] reports that with an echelon he has been able to photograph several new components of a spectral line created by the scattering process. Recently, Meyer and Ramon[4] have published actual photographs of the effect. In contrast with Gross's results they were able to obtain two components only, one at either side of the primary line, which is in accordance with the theory. The shift is approximately 0.06 A. U. and checks satisfactorily with the value calculated from the known velocities of sound in the liquids used. Besides the two components, a central line of unchanged frequency ν_0 appears which may partly be due to unavoidable traces of dust in the liquid, and partly to thermal fluctuations of the index of refraction as a result of molecular rotation, an effect which was not considered in the derivation of Brillouin's theory.

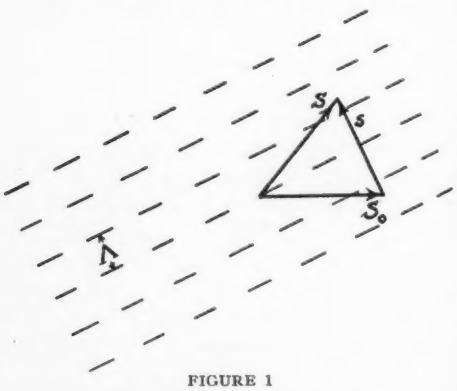

FIGURE 1

2. *Experiments.*—Brillouin[1] mentions the possibility of experimental verification of his calculations, making use of elastic waves set up in a liquid by a quartz crystal driven by a high-frequency oscillator, and it occurred to us that it would be interesting to try the scattering of light by these "artificial" sound waves. The experiment was set up in the following way. In a trough of rectangular cross-section, figure 2, filled with a liquid (benzene, carbon tetrachloride, etc.) a quartz crystal Q was immersed. The leads to the silvered faces of this crystal could be connected with a radio-frequency oscillator. Vibrations set up in the crystal

in this way excite supersonic waves traveling in the liquid in the direction $Q\,E$. Perpendicular to this direction a parallel beam of light from a slit S and a lens L_1 passes through the liquid. The parallel beams, scattered by the illuminated part of the liquid are focussed by a telescope lens L_2. As soon as the crystal is connected to the oscillator, spectra of different orders (like the spectra of an ordinary grating), appear at the left and right of the central image of the slit. The number of orders which can be observed depends on the intensity of the vibrations. Their spacing depends only on the frequency and increases if the frequency of the oscillator is increased.

As in ordinary grating spectra, the blue is the least deviated, and the red the most. With a mercury arc source, the different mercury lines can be observed in every order. Under favorable conditions as to the intensity of the vibration more than 10 spectra to the right and to the left have been obtained. In order to see if the supersonic waves are markedly transmitted by a solid, a glass block of cross-section equal to the trough was immersed in the liquid. Light passed through the liquid in front of and behind the block showed the spectra, but through the block itself produced only the central slit image. We may safely expect, however, that using a higher intensity the effect will also be seen in the solid. No marked decrease in the number of orders visible could be observed in passing the light through portions of the liquid at different distances from the crystal with liquids like benzene or carbon tetrachloride. With glycerine, however, this effect is very pronounced, no doubt due to its high viscosity.

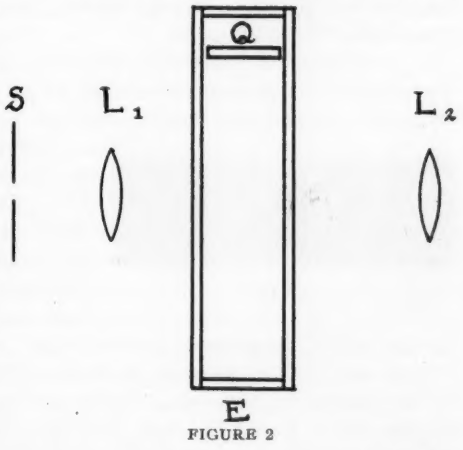

FIGURE 2

Fixing the attention on one of the spectra, preferably of higher order, one can observe that it attains its maximum intensity if the trough is turned through a small angle such that the primary rays are no longer parallel to the planes of the supersonic waves. Different settings are required to obtain highest intensities in different orders. If the trough is turned continuously in one direction, starting from a position which gave the highest intensity to one of the orders, the intensity decreases steadily, goes through zero, increases to a value much smaller than the first maxi-

mum, decreases to zero a second time and goes up and down again passing through a still smaller maximum. The same series of events occurs in turning the trough in the other direction. To make these observations it is necessary to watch the pattern carefully. One thing, however, which can be seen at first glance is the fact that the number of orders visible on the right and the left of the central image is different except for the case in which the primary rays are passing exactly parallel to the planes of the supersonic waves. Turning the trough continuously changes the number of orders to the right or the left in the same way as has been described for the intensity.

The whole effect is rather brilliant and can easily be projected on a screen so as to make it visible to an audience. Figure 3 shows a photograph of the effect obtained in passing monochromatic light of wave-length $\lambda = 5461$ A. U. through toluene, the frequency of the supersonic waves being 5.7×10^6.

3. *Interpretation.*—As a tentative explanation it was first thought that the primary rays passing through the liquid in sheets would, as a consequence of the periodic density variations due to the supersonic waves, acquire phase differences of periodical nature. The light emerging from the grating created in the liquid would show interference patterns of the same general kind as observed with an ordinary grating. The fact that the supersonic waves are not standing waves should make no difference.

FIGURE 3

This explanation, however, fails to explain the observed intensity distribution in the various orders, as can be shown by a more detailed analysis, and a theory has been developed based on the assumption of a volume scattering, in which every volume of the liquid contributes to the total scattering in accordance with Maxwell's equations. In this way, it seems at first, following Brillouin's theory, that one would expect only one reflection for a definite angle of incidence of the primary light and one other reflection on the other side for the same angle taken with negative sign. Moreover, light passing parallel to the planes of the supersonic wave should show no effect at all. These results are evidently at variance with the observations. Taking into account, however, that the dimensions of the illuminated volume of the liquid are finite it can easily be shown that in our case Bragg's reflection angle is not sharply defined and that reflection should occur over a rather appreciable angular range. If l is the length of the path of light in the liquid, Λ the wave-length of the supersonic waves and λ the wave-length of the light, then two quantities are of importance; namely, the quotients l/Λ and Λ/λ. Only if

l/Λ is large compared to Λ/λ does a sharp definition of Bragg's angle exist. Working with a frequency of 10^7 cycles, Λ is about 0.1 mm., l is of the order 10 mm. and λ is about 0.5×10^{-3} mm. In this case, therefore, $l/\Lambda = 100$ and $\Lambda/\lambda = 200$, the quotient of these two quantities is 1/2 and cannot be considered as large. A detailed analysis shows that in such a case reflection will occur over a range of angles left and right of the critical angle which follows from Bragg's relation. Moreover the intensity variations predicted by the theory in varying the angle continuously are just the same as described in relating the rather peculiar experimental results on this point.

We are, however, still left with another difficulty. The theory predicts only the first order spectrum to the right and the first order to the left. But in the theory so far it has been assumed that the variations of the index of refraction are of purely sinusoidal character. If they are not, then we can consider the disturbance as a superposition of variations of frequencies ν, 2ν, 3ν, etc., with the corresponding wave-lengths Λ, $\Lambda/2$, $\Lambda/3$, etc., provided a marked dispersion of the velocity of sound in the frequency region considered does not exist. A departure from sinusoidal character therefore accounts for the existence of the higher order spectra. This departure may be due to the non-sinusoidal cha acter of the crystal vibrations, although higher harmonics may be produced by the scattering itself, if the intensity of the supersonic waves is high enough. This is in agreement with the fact that the higher orders fade out if the intensity of the vibrations of the crystal is decreased.

Adopting the theory, the measurement of the angles θ of the different orders ρ with respect to the central image provides us with a measure of the wave-length of the supersonic wave in terms of the wave-length of the light which has been used. In fact, as in an ordinary grating

$$\sin \theta\rho = \rho\lambda/\Lambda.$$

The frequency can easily be determined with an ordinary wavemeter and so we get a very simple method for the determination of the velocity of sound.

The following table shows some preliminary measurements performed with a very simple spectrometer, together with values calculated from the density and the adiabatic compressibility.

	VELOCITY CALCULATED M./SEC.	FREQUENCY	VELOCITY OBSERVED M./SEC.	FREQUENCY	VELOCITY OBSERVED M./SEC.
Toluene	1290	1.7×10^6	1330	16.5×10^6	1310
Carbon tetrachloride	920	1.7×10^6	940	16.5×10^6	930

Up to now no indication of a change in velocity with frequency has been observed. If it exists, a more careful measurement of the angles for

different orders should show it, as the measurement of say 10 orders simultaneously existing provides us with a frequency range of ν to 10 ν.

[1] L. Brillouin, *Ann. phys.*, **17**, 88 (1922).

[2] A. Einstein, *Ann. Phys.*, **33**, 1275 (1910).

[3] Gross, *Zeit. Phys.*, **63**, 685 (1930); *Naturwiss.*, **18**, 718 (1930); *Nature*, **126**, 201, 400, 603 (1930).

[4] Meyer and Ramon, *Phys. Zeitschr.*, **33**, 270 (1932).

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*THE ADDITIVITY OF THE ENERGIES OF NORMAL COVALENT BONDS**

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The chemical bond between two identical atoms, as in the molecules H_2, Cl_2, etc., may be considered as an example of a normal covalent bond, involving an electron pair shared by the two atoms. The wave function representing this bond cannot necessarily be closely approximated by a function of the Heitler-London type, with the electrons staying on different atoms, but may contain ionic terms, corresponding to the two electrons of the bond on the same atom, the term representing the configuration A^+A^- occurring, of course, with the same coefficient as that for A^-A^+. The contribution of these ionic terms to the wave function for the normal state of the hydrogen molecule has been discussed by Slater.[1]

In the wave function representing the bond between unlike atoms A and B, the ionic terms A^+B^- and A^-B^+ will occur with the same coefficient, of the order of magnitude of those for $A:A$ and $B:B$, if the two atoms have the same degree of electronegativity. We propose to call such a function a normal covalent bond wave function, and the bond a normal covalent bond; and to make the postulate that *the energies of normal covalent bonds are additive*, that is, $A:B = {}^1/_2(A:A + B:B)$, where the symbols $A:B$, etc., mean the energies of the normal covalent bonds. This postulate requires that the energy change for a reaction such as ${}^1/_2A_2 + {}^1/_2B_2 = AB$ involving only normal covalent substances with single bonds be zero. The energy of the normal covalent bond $A:B$ would be given by the integral $\int \psi^* H \psi d\tau$, with ψ the normalized normal covalent wave function. Inasmuch as the energy integral for any wave function for a system must be equal to or greater than the energy of the lowest state of the system, the energy of the actual bond between A and B will either be equal to that for a normal covalent bond $A:B$, or, in case the

bond has ionic character A^+B^- or A^-B^+ in excess of that included in the normal covalent bond, will be greater than this value. Hence, if our postulate is true, actual bond energies will not show negative deviations from additivity; the difference Δ between the actual bond energy and the energy predicted for a normal covalent bond must be zero or positive, and the greater the ionic contribution to the bond, the greater will be the value of Δ.

In Table 1 are given bond energies for hydrogen and the halogens, and their binary compounds, the hydrogen halides and the halogen halides, together with the deviations from additivity. The values of Δ are usually known more accurately than the bond energies themselves because they can be directly measured as heats of reaction. It is seen that the Δ's

TABLE 1

	H:H	F:F	Cl:Cl	Br:Br	I:I
Bond energy	4.44	2.80	2.468	1.962[a]	1.535 v. e.[b]
		HF	HCl	HBr	HI
Actual bond energy		6.39	4.38	3.74	3.07
Predicted from additivity		3.62	3.45	3.20	2.99
Δ		2.77[c]	0.93	0.54	0.08
		ClF	BrCl	IBr	ICl
Actual bond energy		3.82	2.231	1.801	2.143
Predicted from additivity		2.63	2.215	1.748	2.001
Δ		1.19[e]	0.016[d]	0.053[e]	0.142[f]

[a] W. G. Brown, *Phys. Rev.*, **38**, 1179 (1931).

[b] W. G. Brown, *ibid.*, **38**, 709 (1931).

[c] O. Ruff, *Z. anorg. Chem.*, **198**, 375 (1931).

[d] Calculated from unpublished and not final results of Don M. Yost and P. G. Murdoch.

[e] J. McMorris and Don M. Yost, *J. Am. Chem. Soc.*, **53**, 2625 (1931); R. M. Badger and Don M. Yost, *Phys. Rev.*, **37**, 1548 (1931).

[f] J. McMorris and Don M. Yost, to appear in *J. Am. Chem. Soc.* for June, 1932; also W. G. Brown, private communication.

are positive for all of these compounds, which provides strong support for our postulate. Moreover, the values are in agreement with previously formed conceptions of the nature of the bonds in these molecules. A recent discussion[2] of energy curves has shown HF to be largely ionic rather than covalent, while HCl, HBr and HI are mainly covalent, with a small amount of ionic character, decreasing in this order to nearly zero for HI. For HF Δ (= 2.79 v. e.) is nearly as large as the total energy of the normal covalent bond, showing the great influence of the ionic structure H^+F^-. In HCl and HBr the ionic character of the bonds, while much less than for HF, is shown to be important by the values 0.93 and 0.54 v. e. for Δ. HI, with Δ only 3% of the total bond energy, approaches closely the normal covalent type.

The compound BrCl approaches the normal covalent type still more

closely, with a deviation from additivity of less than 1%. The values of Δ for IBr and ICl are also small, but that for ClF is even larger than for HCl, showing that chlorine fluoride is more ionic in character than hydrogen chloride. Chlorine, bromine and iodine do not differ greatly in electronegativity, chlorine and bromine being most similar in this as in other respects (for example, in size, the ionic radii being 1.36 Å for F^-, 1.81 for Cl^-, 1.96 for Br^- and 2.16 for I^-). But fluorine is very much more electronegative than the other halogens, and deserves to be classed by itself as a superhalogen.

It may be mentioned that the property of electronegativity discussed here is closely related to the electronegativity of the chemist, and has little relation to the electron affinity of gaseous atoms.

We may also test the additivity postulate with compounds of a much different type, involving the alkali metals. It is seen from Table 2 that additivity holds to within the experimental error for the molecules KNa and LiH.

TABLE 2

	Li:Li	Na:Na	K:K
Bond energy	1.14[a]	0.76 ± 0.02	0.51 ± 0.02 v. e.
		KNa	LiH
Actual bond energy		0.62 ± 0.05	2.6 ± 0.2 v. e.
Predicted from additivity		0.64	2.79

[a] F. W. Loomis and R. E. Nusbaum, *Phys. Rev.*, **38**, 1447 (1931).

The alkali halide molecules are known to be mainly ionic. The large values of Δ, some of which are given in Table 3, are in agreement with this.

TABLE 3

	KCl	KBr	KI
Actual bond energy	4.5	3.9	3.25 v. e.
Predicted from additivity	1.53	1.24	1.03
Δ	3.0	2.7	2.22

We recognize that the energies of bonds depend on a great many factors, and that additivity of the energies of normal covalent bonds has no rigorous theoretical justification and cannot be expected to hold in all cases; but we feel that the empirical evidence shows that for a number of compounds of varying types the rule is well satisfied, and we believe that it may be usefully applied in the discussion of the nature of the chemical bond, in particular as a criterion for determining the approximation of a bond to a normal covalent bond and as a means of mapping atoms along the scale of electronegativity.

[*] Contribution from Gates Chemical Laboratory, California Institute of Technology, No. 315.

[1] J. C. Slater, *Phys. Rev.*, **35**, 509 (1930).

[2] Linus Pauling, *J. Am. Chem. Soc.*, **54**, 988 (1932).

INVESTIGATIONS IN THE RETENE FIELD. II. alpha-RETENE CARBOXYLIC ACID AND SOME OF ITS DERIVATIVES

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The investigations of Vesterberg,[1] in the case of abietic acid, and of Virtanen,[2] in that of pinabietic acid, have made it clear that both of these important natural products are hydrogenated retene carboxylic acid derivatives. Pinabietic acid, discovered and first studied by Aschan and his co-workers,[3] bears a relationship to abietic acid which has not yet been fully cleared up.[4]

To date the position of the carboxyl group in these acids has not been ascertained. It is important, therefore, to synthesize as many of the retene carboxylic acids as possible, so as to compare their hydro derivatives with these resin acids.

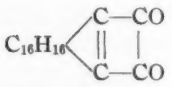
Liebermann and Zsuffa[5] were the first to describe a retene carboxylic acid. They synthesized it by the usual Friedel-Crafts reaction, from retene and oxalyl chloride, and reported its m. p. as 121–123°. It is possible that this m. p. is a misprint for 221–223°.

This work was repeated recently by Komppa and Wahlforss,[6] who obtained an acid m. p. 229–231° (uncorr.) and prepared therefrom the sodium salt, methyl ester and amide, and oxidized it to the corresponding quinone carboxylic acid, thus proving that the carboxyl group was not in either the 9 or the 10 position.

In our previous paper,[7] we described the synthesis of acetylretene from retene and acetyl chloride. We have now succeeded in oxidizing this ketone by sodium hypochlorite or hypobromite to the Komppa and Wahlforss retene carboxylic acid. For convenience, we shall designate this Komppa and Wahlforss acid as the *alpha*-acid, pending the determination of the position occupied by the carboxyl group.

For the firmer establishment of the identity of the two products, several of the derivatives described by Komppa and Wahlforss were prepared from an acid synthesized by their method and also from one obtained by ours. The two series agreed in properties.

In our preparation of the acid by the Komppa and Wahlforss method, we isolated an amorphous intermediate, not mentioned by them, which we failed to obtain in pure crystalline condition, but which yielded the sodium retene carboxylate when treated with a sodium carbonate solution. It seems likely that this intermediate was an oxalyl derivative of type II, rather than type I, since I might be expected to hydrolyze to two different acids, whereas II should yield but one:



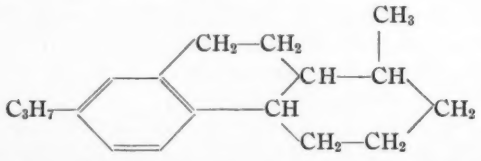
(I)

$C_{18}H_{17}COCOC_{18}H_{17}$

(II)

By reduction of the acid in isoamyl alcoholic solution with sodium, what appears to be an octahydroretene carboxylic acid was obtained in good yield. Its alkaline salts behaved as true resinates. This octahydro acid is now being subjected to catalytic reduction.

The work of Virtanen[2] has shown that when retene is reduced in isoamyl alcoholic solution by sodium, a dihydroretene is produced in which, according to Fogelberg,[8] the hydrogens have entered positions 9 and 10. In the case of our octahydro acid, then, it would seem likely that 2 hydrogens entered at 9 and 10 first, saturating the ethylene bridge, and that this was followed by the complete saturation of one of the adjoining benzene nuclei. The acid would then be a mono-carboxyl derivative of an octahydroretene of formula III:



(III)

Acknowledgments.—Our thanks are due to Dr. Eric Wahlforss, of the Hammermill Paper Co., Erie, Pa., for assuring us that both Professor Komppa and he were quite willing to have us include this acid in the field of our investigations.

We are also indebted to the Trustees of the Joseph Henry Fund of the National Academy of Sciences for financial assistance in meeting the expense of the necessary analytical determinations.

Experimental.—Retene alpha-Carboxylic Acid, $C_{18}H_{17}COOH$.—1. *From Acetylretene.*—To a solution of 5 g. of acetylretene in 200 cc. of methanol, there was added in small portions a hypobromite solution prepared from 50 cc. of 40% sodium hydroxide and 28 g. of bromine. After shaking the mixture for an hour, the excess of hypobromite was destroyed by addition of sodium sulfite. The mixture was diluted and the unoxidized acetylretene filtered out. From the alkaline filtrate, the retene carboxylic acid was precipitated by the addition of dilute hydrochloric acid; yield, 2 g. Purified by repeated crystallization from alcohol, it formed transparent colorless needles, m. p. 237.5–238.5° (corr.).

Anal. Calcd. for $C_{19}H_{18}O_2$: C, 82.0; H, 6.5. Found: C, 82.08; H, 6.51.

Sodium Salt.—Colorless microscopic needles, less soluble in strong

alkali solution than in water; soluble in hot water, but only slightly in cold.

2. *From Retene and Oxalyl Chloride.*—In using this Komppa and Wahlforss[6] method, we made certain changes, the procedure being as follows: To a solution of 20 g. of retene and 47 g. of oxalyl chloride in 300 cc. of carbon disulfide, cooled to 0°, 30 g. of anhydrous aluminum chloride was added. Upon the termination of the main reaction, the mixture was left overnight at laboratory temperature. The carbon disulfide was distilled off, the residue poured into water, a small amount of concentrated hydrochloric acid added and the mixture boiled for 10 minutes. Upon cooling, a dark brown resin separated, which was removed, dried at 110°, pulverized, extracted with ether and the ether evaporated. There remained 16 g. of a yellowish brown resin, which we did not succeed in crystallizing. It dissolved completely in a boiling 10% sodium carbonate solution and on cooling there separated the sodium salt of retene alpha-carboxylic acid, identical in appearance with the salt from our acid; yield, 12 g.

This salt was suspended in cold water and dilute hydrochloric acid was added. The precipitated retene carboxylic acid was collected, dried at 110°, and purified by crystallization from absolute alcohol, acetone and finally benzene. There resulted small transparent colorless needles, m. p. 237.5–238.5° (corr.), which m. p. was not altered by admixture with the acid prepared from acetylretene.

Anal. Calcd. for $C_{19}H_{18}O_2$: C, 82.0; H, 6.5. Found: C, 81.81; H, 6.31.

Komppa and Wahlforss, as noted above, reported a m. p. of 229–231° (uncorr.).

Methyl Ester.—This ester, prepared either from our acid or from that of Komppa and Wahlforss, by the action of methyl sulfate upon the sodium salt, crystallized from acetone in pale yellow scales, m. p. 96–97° (corr.), as recorded by Komppa and Wahlforss, and an intimate mixture of the two products melted at the same point. The ester obtained from our acid, as a further check, was analyzed.

Anal. Calcd. for $C_{20}H_{20}O_2$: C, 82.2; H, 6.9. Found: C, 81.96; H, 6.68.

Anilide, $C_{18}H_{17}CONHC_6H_5$.—A mixture of 1 g. of retene-alpha-carbonyl chloride, $C_{18}H_{17}COCl$, prepared as described by Komppa and Wahlforss,[6] with 10 cc. of aniline, was refluxed for 10 minutes, cooled and poured upon ice to which some hydrochloric acid had been added. The precipitated anilide was filtered out, washed with dilute hydrochloric acid, then with water, dried and crystallized from alcohol. Colorless, lustrous flakes were obtained, m. p. 224.5–225.5° (corr.), which gradually turned brown on standing; yield, nearly that calculated. For the preparation of this

anilide, we used two different lots of retene carboxylic acid, one prepared by the Komppa and Wahlforss method and the other from acetylretene. The anilides so obtained were identical, possessed the same m. p. individually or when mixed.

Anal. Calcd. for $C_{25}H_{23}ON$: C, 85.0; H, 6.5. Found: C, 85.02; H, 6.78.

Retenequinone alpha-Carboxylic Acid, $HOOC.C_{16}H_{15}\begin{matrix}CO\\|\\CO\end{matrix}$.—Komppa and Wahlforss, who obtained this acid by oxidation of the sodium retene carboxylate with chromic oxide in glacial acetic acid solution, described it as dark red needles, m. p. about 237–240°, and analyzed it by titration with $N/100$ potassium hydroxide in alcoholic solution.

We prepared it similarly, using the free retene carboxylic acid, instead of its sodium salt, and the product decolorized and crystallized from glacial acetic acid, formed orange needles, which decomposed above 252°; yield, 0.4 g. from 0.5 g. of the retene carboxylic acid.

Anal. Calcd. for $C_{19}H_{16}O_4$: C, 74.0; H, 5.2. Found: C, 73.78; H, 5.38.

The product dissolved in concentrated sulfuric acid to a green solution whose color was discharged by dilution.

Methyl Ester.—This was prepared, like the corresponding acid, by oxidation of methyl retene carboxylate (2 g.) with chromic oxide (4 g.) in glacial acetic acid solution (20 cc.), and formed orange needles which were purified by crystallization from alcohol, and then melted with decomposition at 197.5–198.5° (corr.); yield, 1.7 g.

Anal. Calcd. for $C_{20}H_{18}O_4$: C, 74.5; H, 5.6. Found: C, 73.81; H, 5.74.

In concentrated sulfuric acid, it dissolved to a green solution, whose color disappeared upon the addition of water.

Retophenazine alpha-Carboxylic Acid.—$HOOCC_{16}H_{15}\begin{matrix}C:N\\|\\C:N\end{matrix}C_6H_4$.—To a boiling solution of 0.5 g. of retenequinone carboxylic acid in 10 cc. of glacial acetic acid, there was added 0.7 g. of a solution of *o*-phenylenediamine HCl in a small quantity of alcohol and then 0.5 g. of anhydrous sodium acetate in small portions. After refluxing the mixture for 15 minutes, it was allowed to cool. The small yellow needles which separated were recrystallized from glacial acetic acid and then decomposed above 272°. They dissolved but slightly in glacial acetic acid and were nearly insoluble in all other solvents. Their analysis proved very difficult, as the figures for carbon show.

Anal. Calcd. for $C_{25}H_{20}O_2N_2$: C, 78.9; H, 5.3; N, 7.4. Found: C, 77.44, 77.28; H, 5.00, 5.51; N, 7.8.

In concentrated sulfuric acid it formed a cherry-red solution, the color of which was discharged when water was added.

Methyl Ester.—A solution of 0.5 g. of methyl retenequinone carboxylate in 10 cc. of glacial acetic acid was treated with 0.7 g. of *o*-phenylenediamine HCl and 5 g. of anhydrous sodium acetate, and the crude product recrystallized from the same solvent. Fine yellow needles resulted, m. p. 187–188.5° (corr.); yield, 0.3 g.

Anal. Calcd. for $C_{26}H_{22}O_2N_2$: C, 79.2; H, 5.6. Found: C 78.78; H, 5.47.

In concentrated sulfuric acid, the solution was purple, which color was discharged by dilution.

Octahydroretene alpha-Carboxylic Acid, $C_{18}H_{25}COOH$.—To a boiling solution of 2 g. of sodium retene alpha-carboxylate in 25 cc. of isoamyl alcohol, 2 g. of sodium was added in small portions. Upon the conclusion of the reduction, water was added, and the aqueous and amyl alcohol layers separated. Acidification of the aqueous layer showed that it was practically free from the octahydroretene carboxylate. The amyl alcohol layer was therefore subjected to steam distillation, to remove the amyl alcohol. The residue in the distilling flask was filtered from some suspended tar, the aqueous solution acidified, and the precipitated octahydro acid crystallized repeatedly from absolute alcohol. It formed small colorless prisms, m. p. 181–182° (corr.); yield, 1.2 g.

Anal. Calcd. for $C_{19}H_{26}O_2$: C, 79.7; H, 9.2. Found: C, 79.98, 80.28, 79.73, 79.71; H, 8.62, 9.47, 9.19, 9.34.

The sodium salt dissolved in hot water, but not in aqueous alkali. This acid and its derivatives are now being investigated further.

Summary.—1. By the oxidation of acetylretene with sodium hypobromite, a retene carboxylic acid is obtained which is identical with the acid synthesized by Komppa and Wahlforss from retene, oxalyl chloride and aluminum chloride.

2. Various new derivatives of this acid are described.

3. Reduction of the acid by sodium in isoamyl alcoholic solution yields an octahydroretene carboxylic acid. This is the nearest approach so far achieved synthetically to a true aporesin acid of the abietic series.

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[3] (*a*) Aschan, *Finska Kemistamfundets Medd.*, April, 1917; (*b*) Aschan and Ekholm, *Ann.*, **424**, 117 (1921).

[4] (*a*) Aschan, *Ber.*, **55**, 2944 (1922); (*b*) Ruzicka, Goldberg, Huyser and Seidel *Helv. Chim. Acta*, **14**, 545 (1931).

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CHANGES IN THE CELLS OF SPIROGYRA ASSOCIATED WITH THE PRESENCE OF WATER POLYMERS

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In a preliminary note the junior author (T. C. Barnes, 1932) has reported a striking difference in the general appearance of masses of *Spirogyra* filaments maintained in ice water renewed daily and in water from recently condensed steam. The working hypothesis was suggested that the degree of polymerization of the water molecules was in some way associated with the phenomena observed. Since the time of Röntgen (1892) it has been recognized that water is a highly complex system consisting of several polymers of hydrogen oxide. The simple H_2O of hydrol is represented by dry steam. Ice may be regarded as $(H_2O)n$, perhaps trihydrol (H. T. Barnes, 1928), and liquid water is probably dihydrol mixed with smaller quantities of monohydrol and trihydrol. The chemical activity of water appears to be associated with the colloidal form, trihydrol, which, according to Sutherland (1900 and later papers) is present in large quantities even at relatively high temperatures. For example, at 20°C., 31.1% of the system may be in the form of liquid ice.

The present report, dealing with the effect of specially prepared ice water and recently condensed steam upon the cells of *Spirogyra nitida*, is based on experiments carried on in the Botanical Laboratory at McGill University during December, 1931, and in the Osborn Zoölogical Laboratory, Yale University.

Every precaution was taken to eliminate impurities in the water. Silica and Pyrex containers were used throughout and the steam was condensed in a silica still from twice-distilled water of great purity. Two 100-watt Mazda bulbs provided a continuous source of illumination 40 cm. above the cultures in a subterranean constant temperature room. The constant flooding of the cultures by light was a factor tending to hasten the destruction of trihydrol, for illumination is a recognized method of ice prevention in modern ice engineering (H. T. Barnes, 1928). The air temperature of the room, 22°C., also curtailed the life of the colloidal water molecules. In the preliminary note (T. C. Barnes, 1932) it was reported that colloidal water appeared to have an accelerating effect on the growth of mass cultures of *Spirogyra* maintained at 10° in daylight. It is therefore of great interest that the trihydrol effect was secured again in the present experiments under different conditions of temperature. The trihydrol solutions were renewed each day from frozen silica-distilled water and

natural ice from the St. Lawrence River kindly furnished by Dr. W. H. Barnes of the Department of Chemistry. This clear ice formed over running water is the purest substance found in nature and conductivity and bacteriological tests have been made on water from this ice (H. T. Barnes, 1926, p. 436); in fact Zsigmondy (1920) recommends melted ice as the purest water for microscopical work, and H. T. Barnes (1928) has pointed out repeatedly that the best conductivity water prepared by distillation methods is not superior in purity to that from natural ice formed over running water. Each day the old trihydrol water was decanted and 50 cc. of fresh melted ice water at 10° was added which soon reached room temperature in the quartz beakers. The water containing larger proportions of monohydrol was condensed from steam in a water jacket at 10° and was at a temperature (24°C.) slightly above that of the room when added to the cultures. It was added to the cultures immediately after condensation.

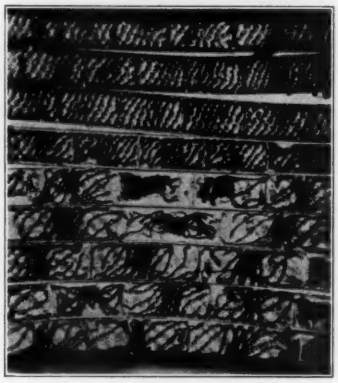

FIGURE 1

Microphotograph of *Spirogyra* cells influenced by water polymers. Above: four filaments from ice water cultures. Below: five filaments from water containing less trihydrol. See text.

Six series of experiments were carried out involving over one hundred filaments of *Spirogyra*. Series 1 contained no salts and was run for 16 days, each beaker containing 40 cm. of filament. Series 2 contained 5 cc. of 0.6% Knopf's solution added each day to the renewed 50 cc. of water and was maintained for seven days. Each beaker contained a mass culture of approximately $2^1/_4$ cc. of *Spirogyra*. In Series 3 no salts were present; a single filament, 20 cm. long, was placed in each of the cultures which were under observation for four days. The fourth series was like the first but 5 cc. of Knopf's solution were added and the effect secured in less than two days. Series 5 was a duplicate of Series 1, lasting for three days at the end of the time interval for the first.

Trihydrol water was also made by freezing condensed steam and the biological results secured in Series 4 and 6. In Series 6 short filaments from the same culture of *Spirogyra* were used and each cell was measured daily.

It seems unnecessary to give a complete daily record of observations for each of the cultures. In the first series on Dec. 27 (eighth day) the naked eye could discern a beaded appearance in the monohydrol cultures while the filaments in trihydrol appeared uniformly green and quite normal. Microphotographs (Fig. 1) and microscopical observations were

made the next day. The difference in the microscopical appearance of the cells in the two types of water was very striking. The cells in trihydrol looked for the most part normal; their osmotic pressure was normal as judged by the convexity of the end cells. In most cases the individual cells were shorter than normal, indicating that cell division had intervened. A few cells, however, showed incipient contraction of the chloroplast from the wall layer, perhaps due to mechanical disturbance. It was noted that during the interval following photography, many more cells displayed contraction. In some cases a still incomplete transverse cell wall was observed and the chloroplast had not yet divided. It appeared that these cells in trihydrol were more sensitive than normal to mechanical disturbance.

At the same time observations were made on the cells of the same experiment exposed to water richer in mono- and di-hydrol. Most of the cells were completely dead and in fact must have been dead for some time. In many cases the chloroplasts were entirely decolored and in all the cells the protoplast and chloroplast were more or less completely shrunken. There was no question of their complete death in a manner characteristic of *Spirogyra*. The cells were of normal size and it is doubtful whether cell division had occurred but of this we are not sure. An occasional cell still showed a rather high osmotic pressure, perhaps even normal osmotic pressure, as indicated by the convex ends abutting on dead cells. However, these cells were clearly abnormal as shown by irregularities in the chloroplastids and their massing in the center of the cells.

On January 2 the trihydrol cultures were examined again under the microscope and were found to contain a mixture of dead and living cells. The majority of cells were alive and quite normal. A good deal of abscisson (cf. Lloyd, 1926) had taken place. Of course it was not expected that *Spirogyra* would live indefinitely in pure water without salts, even in the presence of trihydrol. The filaments in the cultures lacking trihydrol were nearly all dead, with a few living cells present. The living fragments had all abscissed.

The third series afforded the most interesting features and will be selected for discussion. At 4:00 P. M. on December 29 filaments of uniform and normal appearance were placed in ice water, in condensed steam and in pond water (control), and microscopical observation revealed the trihydrol effect within 44 hours. A microphotograph of a pair of filaments from each culture is presented in figure 2. It will be seen that the cells of the control are quite normal. Those in trihydrol, on the other hand, although not quite normal due to the absence of salts, were quite vigorous with the chloroplasts full of starch and crowded at the middle. The presence of colloidal water had stimulated cell division as revealed by the great number of new transverse walls in the filaments. In the mono-

hydrol water the cells were, for the most part, dead. The protoplasts were greatly shrunken but some cells were alive but succumbed quickly when disturbed. The living cells were much longer than the dead ones, indicating that although some growth had intervened, cell division was unable to take place in the monohydrol. For example, two cells end to end in the control measured 55 microns while two of the elongate monohydrol cells measured as much as 80 microns. Further details may be ascertained from the photograph (Fig. 2).

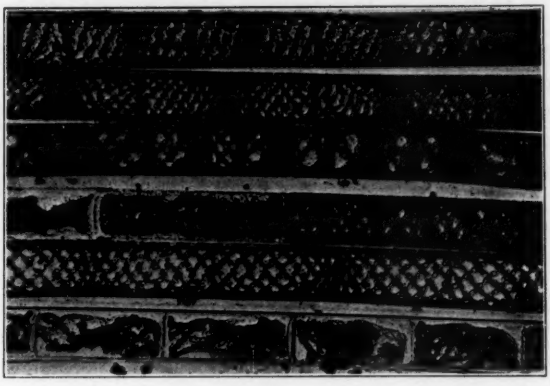

FIGURE 2

Microphotograph of *Spirogyra* cells influenced by water polymers.

A pair of filaments was taken from each culture. Pair at the top: control cells from pond water. Middle pair: filaments from water rich in trihydrol. Note that many transverse walls are present indicating that cell division has taken place. Pair at bottom: cells exposed in water consisting of mono- and dihydrol. Note that many cells are dead and in the living filament growth has occurred without cell division.

Finally we desire to mention the results secured in the cultures which contained, besides the various polymers of water, nutritive salts.

In Series 4 which was maintained for two days, two kinds of trihydrol solutions were prepared, one from clear river ice, and one from frozen silica-distilled water. Both contained 5 cc. of 0.6% Knopf's solution, which was replaced each day when the 50 cc. of water was changed. Both these colloidal water cultures preserved the filaments in a healthy condition and in each a new effect appeared. The filaments became tightly curled, due, no doubt, to the presence of the nutritive salts. This was probably an osmotic phenomenon, and, at all events, indicated a high state of vigor.

The filaments of *Spirogyra* exposed to monohydrol in the same experiment afforded a completely different microscopical picture. In the cultures lacking nutritive salts it was observed that nearly all the cells were dead and the surviving filaments were distinctly abnormal. In the monohydrol cultures to which 5 cc. of 0.6% Knopf's solution had been added the absence of the colloidal water was quite evident in spite of the fact that the nutritive salts were introduced at the same time that all the solutions were regularly changed. For the most part the cells were dead, in a manner characteristic for *Spirogyra*. Very few living filaments were detected and all of these were in an abnormal condition. It was estimated that approximately 10% of the cells were still alive. In the monohydrol cultures lacking Knopf's solution almost all the cells were dead.

The results secured in Series 4 in which the cultures contained Knopf's solution show clearly that we are not dealing with the familiar toxic effect of distilled water for it is quite evident that the degree of polymerization of the water exerts a profound physiological effect even when the same nutritive salts are present in all the solutions.

In Series 6 in which the trihydrol water was prepared from condensed steam the length of each cell was measured daily. Small Petri dishes of 10-cc. capacity were used each containing 6 cc. of the solutions. The effects described above were again secured. In these short filaments 9 to 12 cells in length, however, the quantitative data show that cell division occurred in the control only (cells 5, 7 and 9). It was also observed that growth occurred in the trihydrol water. The descriptions of the protoplasts were the same as those for the cultures mentioned above. The death of the cells in monohydrol water was a better indicator than cell length.

Experiments are now under way in which water vapor pressures and CO_2 and O_2 content of the water are being varied. In the above experiments the water was sprayed into the cultures with the narrow nozzle of a pipette and it is probable that this allowed attainment of equilibrium in the gas content of the water. The wide extent of surface of the shallow solutions also permitted rapid attainment of equilibrium. In the experiments now under way evidence is at hand that gas content and vapor pressure are factors of minor importance.

It is not generally known that the attainment of equilibrium of the polymers of water is slow (H. T. Barnes, 1930). In fact the passive state of dihydrol water is now explained on the ground that time is required for the restoration of the trihydrol in the solution.

In conclusion it may be said that the explanation of the trihydrol effect must remain a matter of speculation at the present time and it seems unprofitable to propose *ad hoc* hypotheses before more research is com-

pleted. In this connection one may mention the well-known chemical inertness of water lacking trihydrol, the possibility that the colloidal water is utilized in photosynthesis, and the possible significance of the higher surface tension of trihydrol water. The results must speak for themselves until they are supported by an adequate explanation based on the physical properties of water polymers.

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CHARACTER RECOMBINATION IN DROSOPHILA

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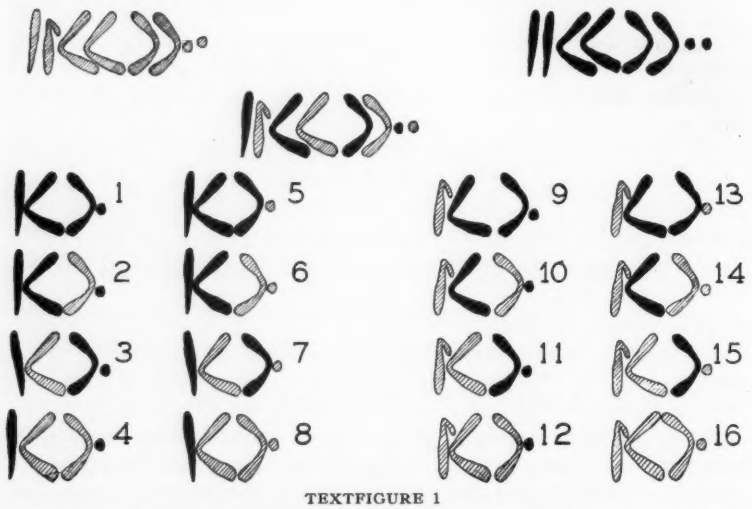
Strictly speaking, recombination of genes cannot be compared (as it sometimes is in elementary text books) to "recombination of beads out of a barrel." According to current theories it is more properly to be compared to recombinations of *strings of beads*, the length of the string depending upon the length of the cross-over segment and the number of strings depending upon the number of such segments. These two values are known to vary between very wide limits. The purpose of this note is to call attention to the fact that the effect of the length and number of "strings" upon character recombination should be morphologically demonstrable.

In the case of the male of *Drosophila melanogaster*, with no crossing-over and only four pairs of chromosomes, recombination should be almost at a minimum, since such an individual can give rise to only sixteen genetically different types of sperm. The experiment should be relatively simple and may be outlined somewhat as follows (see the accompanying diagram).

The experiment is begun with two strains of *Drosophila* which we may for convenience designate as the gray race and the black race. They can

be any two races, though the more multiple factor differences between them, the more readily can the results be classified. (For that matter they may also differ by known genes, but that is not necessary for the success of the experiment.) Crossing the two races will produce F_1 males of the type illustrated in the diagram. These F_1 males, according to current theories, should produce only the 16 types of sperm illustrated in the diagram. Of these 16, eight will be female producing (nos. 1 to 8) and eight will be male producing (nos. 9 to 16). Mated with females of the gray race or of the black race, only 16 classes of offspring should appear, and they should be in approximately equal numbers. It should not be difficult to identify the eight classes of males and eight classes of females in each back-cross. One



TEXTFIGURE 1

The sixteen genetically different types of sperm produced by an F_1 male of *D. melanogaster*. Further explanation in text.

type should be like the parent race, another should be identical with the F_1. Furthermore since the fourth chromosome is so much smaller it might be expected to play a much less important rôle and as a result we would expect four main types of back-cross females and four of back-cross males. Even though the races employed in the experiment differed by no genes with conspicuous effects, it should be possible to identify the classes, for if the two races differed by ten thousand small modifying genes, there should still be only 16 classes. Even though the back-crosses were raised by the tens of thousands they should still fall into only 16 classes of approximately equal size.

Once the classes are identified it will be possible to make a number of interesting comparisons. In the back-cross to the gray race, the black chromosomes can be compared one at a time, in their effect on the gray complex. The effect of a black first chromosome and of a black second chromosome, singly and together, can be traced upon every measurable character of the offspring. It would furthermore be possible to make a very delicate test of the effect of the cytoplasm upon different characters. Sperm of type No. 1, in crosses back to the black race, should produce individuals which are chromosomally identical with the black race but which have some gray cytoplasm. A similar comparison can be made between the back-cross individuals produced by sperm No. 16 and the gray race. Even more interesting in this connection would be the comparison of the three following groups: sperm No. 1 × gray; sperm No. 16 × black; the original F_1. These three groups should be chromosomally identical though differing widely in the cytoplasmic contributions of black and gray. It might in this way be possible to demonstrate any hitherto undetectable effect of the cytoplasm.

These are but a few of the unusual opportunities offered by such an experiment and it is hoped that some geneticist with the necessary facilities will carry it through. There are a number of interesting theoretical considerations in the case of the male of *D. melanogaster*. The number of types of gametes produced by any normal diploid should roughly be equal to 2^n, where n equals the number of crossover segments. In the male of *D. melanogaster*, this will be 2^4 or 16 as we have seen above. In the female it will be 2^n, with n supposedly quite a large number. It is interesting to compare the number of genetically different zygotes per mating in the case of *D. melanogaster* ($2^n \times 2^4 = 2^{n+4}$) and in any species in which crossing-over takes place in both sexes ($2^n \times 2^n = 2^{2n}$). If n is at all large the number of genetically different zygotes per mating must be enormously greater (2^{n-4} times as great) in species in which crossing-over occurs in both sexes. From these purely theoretical considerations it would seem that a low intra-family variability should be morphologically demonstrable in *D. melanogaster*.

EFFECT OF TEMPERATURE ON THE RATE OF CHANGE OF THE UNSTABLE MINIATURE-3 GAMMA GENE OF DROSOPHILA VIRILIS

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Communicated May 15, 1932

It has been shown by Muller[1] and later by Goldschmidt[2] and by Jollos[3] that high temperature increases the mutation rate of genes of *Drosophila melanogaster*. It was of interest, therefore, to ascertain what effect the temperature has on the rate of change of the unstable miniature gene.

Material.—Miniature-3 gamma was selected as the most suitable material for measuring the temperature effect since this gene is unstable in somatic cells only and in addition to that changes with a relatively low rate. Changes from the miniature gene to the wild-type gene manifest themselves as wild-type regions in miniature wings. The frequency of the change is such that between five to ten per cent of flies may be expected to have mosaic wings. This frequency is low enough so that two wild-type spots rarely occur on the same fly and the chances of two spots overlaping are negligible.

The line used in experiments was highly inbred. Its history is as follows: about 50 generations of mass matings, two generations of pair matings, ten generations of mass matings, one generation of pair mating, two generations of mass matings, one generation of pair mating and finally two generations of mass matings. Since the gamma gene is capable of changing into an alpha or a beta gene, both of which differ from gamma in their stability, the line has been tested whenever it was propagated by pair matings and only flies having gamma genes were used to continue the line.

Methods.—Since the gamma line was inbred for about 68 generations it could be expected that the flies were genetically homogeneous. To eliminate the effect of mutations, which might possibly have occurred in the line, offspring from mass matings were used in the experiments. Four sets of 25 pairs of flies, all taken from the same culture bottle, were used as parents. These sets were numbered 1, 2, 3 and 4, respectively. Parent flies were allowed to lay eggs for 24 hours and were then transferred into fresh culture bottles. This process was continued every 24 hours until 33 transfers were made giving a total of 132 culture bottles. A part of these was then kept at 20 ± 0.2 degrees centigrade, another part was kept at 25 ± 1 degrees centigrade and the remaining part at 30 ± 0.2 degrees centigrade. Thus the eggs from the same set of parents

were allowed to hatch and develop into flies at three different temperatures.

Wings of the newly hatched flies were examined under 40.8× magnification for the wild-type regions. By using weak light reflected from the sub-stage mirror through the wing it has been possible to detect minute wild-type spots.

As already mentioned only in a very few instances more than one wild-type area has been found on one fly. In these cases each wild-type area was considered as a separate mosaic.

Results.—In table 1 summaries of results are given. It is evident from

TABLE 1

NUMBER OF MOSAIC FLIES IN MINIATURE-3 GAMMA CULTURES REARED AT DIFFERENT TEMPERATURES

Set	Number of cultures	Mosaic		Miniature		Total		Per cent of mosaics		
		♀	♂	♀	♂	♀	♂	♀	♂	Total
		Temperature 30 ± 0.2 Degrees Centigrade								
1		45	43	410	409	455	452	9.89	9.51	9.70
		53	51	607	612	660	663	8.03	7.69	7.86
		70	72	697	733	767	805	9.13	8.94	9.03
4	11	41	39	353	335	394	374	10.41	10.43	10.42
	51	209	205	2067	2089	2276	2294	9.18 ± 0.45	8.94 ± 0.44	9.06 ± 0.35
		Temperature 25 ± 1 Degrees Centigrade								
1	4	34	41	280	252	314	293	10.83	13.99	12.36
2	4	37	26	273	245	310	271	11.94	9.59	10.84
3	4	25	35	268	256	293	291	8.53	12.03	10.27
4	4	25	21	209	196	234	217	10.68	9.68	10.20
	16	121	123	1030	949	1151	1072	10.51 ± 0.56	11.47 ± 0.93	10.98 ± 0.47
		Temperature 20 ± 0.2 Degrees Centigrade								
1	7	41	41	376	371	417	412	9.83	9.95	9.89
2	7	49	55	353	358	402	413	12.19	13.32	12.76
3	7	59	73	491	468	550	541	10.73	13.49	12.10
4	7	46	47	437	449	483	496	9.52	9.48	9.50
	28	195	216	1657	1646	1852	1862	10.53 ± 0.51	11.60 ± 0.54	11.07 ± 0.41

that table that of the flies reared at 30 degrees centigrade 9.06 ± 0.35 per cent were mosaics, of the flies reared at 25 degrees centigrade 10.98 ± 0.47 per cent were mosaics and of those reared at 20 degrees 11.07 ± 0.41 per cent were mosaics. The difference in the percentage of mosaics is 1.92 ± 0.58 between 30 and 25 degrees, 2.01 ± 0.54 between 30 and 20 degrees and 0.09 ± 0.62 between 25 and 20 degrees. The value for the difference divided by its probable error is 3.3 in the first case, 3.7 in the

second case and 0.15 in the third case. In the first two cases the difference is significant and in the third case it is not.

Discussion.—Temperature Effect.—There is very little doubt that among the flies reared at 30 degrees centigrade significantly fewer mosaics were observed than among the flies reared at either 25 or 20 degrees. The question arises, however, whether that difference is due to temperature or whether it is a result of some other cause.

The flies reared at 30 degrees are appreciably smaller than the flies reared at either 25 or 20 degrees. The size of the wings of these flies is about half of the size of the wings of the flies raised at 20 degrees. In addition to that the wings of the 30 degree flies have a tendency to wrinkle which tendency is accentuated in cultures having even a slight excess of moisture. It is very probable, therefore, that small and wrinkled wings are responsible for the smaller number of observed mosaics in the 30 degree cultures, since the minute wild-type spots are easily missed on such wings. If the smaller number of mosaics were due to temperature it could be expected that the flies reared at 25 degrees would have the number of mosaics intermediate between the flies raised at the 20 and 30 centigrades, which, however, has not been observed. The explanation appears feasible, therefore, that the lower number of mosaics observed in the 30 degree cultures is due to an observational error rather than to the effect of temperature.

However, before reaching the conclusion that ten degrees difference in temperature does not affect the frequency of change of the miniature-3 gamma gene it is necessary to consider another factor in which the cultures raised at different temperatures differed. That factor is the time which it takes for a fly to develop from an egg to an imago. At 30 degrees centigrade this period is 11 days and at 20 degrees it is 19 days. It is evident that if the rate of the change in the gene were a function of time then the possible increase due to high temperature might have been obscured in our experiment. Another experiment was performed, therefore, to clear up this point. Flies were raised at 30 degrees but the period of the development was prolonged by crowding which was accomplished by using small culture bottles. In this experiment, also, 25 pairs of flies were allowed to lay their eggs for a period of 24 hours and then they were transferred into another set of bottles. The flies began to hatch on the thirteenth day and continued hatching for fourteen days. The flies in the previous experiment began to hatch on the eleventh day and all hatched within four days. Of the 175 flies which hatched after 13 to 17 days from the time the eggs were laid, 17 or 9.71 per cent were mosaics and of the 82 flies which hatched from the same two cultures after 18 to 24 days from the time of the egg laying, 7 or 8.54 per cent were mosaics. It is evident, therefore, that the change in the gene is not a function of

time and that the inequality in the duration of the development from an egg to an imago has not affected the results of the temperature experiment.

The experiment, therefore, indicates that ten degrees centigrade difference in temperature has not affected the rate of change in the unstable miniature-3 gamma gene in a detectable degree. The question now arises how this finding can be brought in accord with observations of the others, namely, that the high temperature increases the mutability of genes. As a possible explanation the following working hypothesis might be offered. Studies made with the unstable rose flower color of Delphinium[4] and with the unstable chlorophyll color of Polystichum[5] indicate that the change in the gene occurs at the time of cell division. It seems probable, therefore, that this change is caused by the failure of the gene to reproduce itself exactly, viz., that the new gene formed next to the old one differs from the old gene. If a gene is considered to be a large organic molecule the changed gene may differ only in the position of groups inside that molecule (isomere). The characteristic of an unstable gene, then, would be that a shift in the molecular organization of the gene frequently occurs during gene reproduction. That process, apparently, is not affected by high temperature. A similar shift in the molecule organization might, however, be produced by environmental agencies such as x-rays and high temperature. As shown in the x-ray work the change in the gene may be produced when cells are not dividing as well as when they are dividing. X-rays, therefore, can accomplish the molecular rearrangement in a molecule already formed. If it is assumed that a similar effect could be produced by high temperature then the explanation of our results becomes evident. In the case of miniature-gamma certain percentage of new genes having gene molecule arranged differently from that of the parent gene is formed during the reproduction of the gene. This process is characteristic of the unstable gene and is not affected by the temperature. Identical rearrangement in gene molecule could probably be produced by high temperature in a gene already formed. The work of Muller and others indicate that the rate of this change for any one particular gene would be so low that it could not have been detected in our experiments.

The Effect of Sex on the Instability of the Gene.—Another point brought out in this experiment is the effect of sex on the instability of miniature-3 gamma gene. Since this gene is sex-linked every female carries approximately twice the number of genes carried by a male. If the rate of instability were the same in both sexes about twice as many mosaic spots should be found in females as in males. The data in table 1 indicate that mosaic spots occurred in both sexes with the same frequency. This means that the rate of instability of the gene is about twice as high in males as it is in females.

It is known that the rate of instability of the gamma gene can readily

be influenced by several genetic factors.[6] The higher rate in the males might be accounted for by the assumption that the male sex stimulates the instability. Experiments are now in progress which are expected to throw more light on this problem.

Summary.—Miniature-3 gamma gene is unstable in somatic cells. The results of experiments in which the flies carrying this gene were reared at 30 ± 0.2, 25 ± 1 and 20 ± 0.2 degrees centigrade, respectively, are interpreted to indicate that these temperature differences have not produced any effect on the instability of the gene.

It has been found that the frequency of gene changes was alike in males and in females in spite of the fact that a female carries approximately twice the number of miniature genes carried by a male.

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AN INTERCHANGE IN MAIZE GIVING LOW STERILITY AND CHAIN CONFIGURATIONS

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The culture of maize which gave semisterile-1 plants (Brink, 1927) contained also two plants with a low percentage of pollen abortion (10–18 per cent based on counts made at that time). This culture had not been x-rayed. Low steriles appeared in the first generation crosses obtained from one of these plants. Pollen counts show them to be about 25 per cent sterile. Thirty-seven plants were counted, giving a total of 58,000 pollen grains, of which 26.3 per cent were practically devoid of starch.

Root tip counts showed that the low sterile plants have 20 chromosomes, demonstrating that this low sterile line differs from the 21 chromosome low steriles arising from irregular (3–1) disjunction of the chromosomes in the rings of semisteriles (Burnham, 1930, McClintock, 1931).

Diakinesis configurations in the microsporocytes of low sterile plants showed 8 bivalents plus a chain of four chromosomes, while their normal sibs had 10 bivalents. In the low sterile plants, a chain of four chromo-

somes is formed regularly. No case has been observed of a ring of four, nor any breaking of the chain to form "pairs."

The chain is associated with the nucleolus. This indicates that the satellite chromosome is involved, since in *Zea mays*, this chromosome is the only one which is attached to the nucleolus.

In order to identify the other chromosome involved in the chain, the low sterile was crossed with homozygous interchange types in which the chromosomes involved had been determined. The low sterile was used as the pollen parent to avoid any complication from $n + 1$ gametes which sometimes are formed from a 3–1 disjunction of four associated chromosomes. It has been found from a study of trisomic plants that $n + 1$ pollen functions only rarely in competition with n pollen (McClintock and Hill, 1931). Crosses of the low sterile with semisterile-1 (an interchange between the *P-br* and the *B-lg* chromosomes, Brink and Cooper, 1931) gives a chain of 6 chromosomes plus 7 bivalents, indicating that one of these two chromosomes is involved. The cross with semisterile-3 (an interchange between the *P-br* and the gl_1-*ra* chromosomes, unpublished data of the author) also gives a chain of 6 chromosomes, indicating that one of these two is concerned. Since the *P-br* chromosome is the one common to semisterile-1 and semisterile-3, it must be the one involved in the low sterile.

The chain of 4 in the low sterile therefore involves the satellite chromosome, carrying the genes of the *Y-pl* linkage group (McClintock, 1932); and the *P-br* group which belongs to the longest chromosome (Burnham, 1930).

In order to obtain evidence regarding the nature of the chromosomal change, pachytene stages in microsporocytes of the low sterile plants were studied. McClintock, 1930, and Brink and Cooper, 1931, have shown that the chromosomes at this stage are very much longer than at diakinesis and are closely synapsed. The interchanges give a cross-shaped synaptic complex resulting from synapsis of homologous parts.

The pachytene configuration in the low sterile plants is definitely not a cross-shaped complex but approximates a T shape.

The normal satellite chromosome is represented in figure 1*a*. At the pachytene stage, according to McClintock's (1931 and unpublished) description, it consists of a long arm beyond the spindle fibre insertion region, a short section from the insertion region to an enlarged reticulate region, followed by a very lightly stained, almost colorless region, then the satellite proper. The "colorless" and reticulate regions are attached to the nucleolus. The satellite proper is composed of 4 chromomeres, i.e., a basal chromomere adjacent to the "colorless" region, two small intermediate chromomeres, and a much larger distinctive terminal chromomere (see *a*, Fig. 1). In many figures the two intermediate chromomeres are so close together that they appear as one.

The *P-br* chromosome has almost a median insertion region (Fig. 1*b*). The relative length of the two arms is 1:1.2+ (McClintock, 1929, and from prophase measurements in these studies). In the pachytene stage, the arms can be distinguished readily, since on the short arm the region adjacent to the insertion appears to be more darkly stained.

In the low sterile plants, the pachytene figures clearly indicate that an

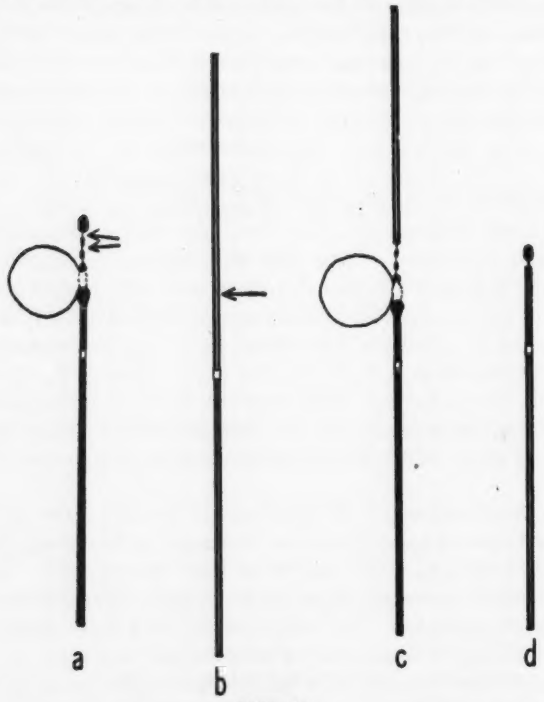

FIGURE 1

a—normal satellite chromosome; *b*—normal *P-br* chromosome; *c*, *d*—the new chromosomes resulting from an interchange at the positions of the arrows in *a* and *b*. The end of the satellite has interchanged with a piece of the longer arm of *P-br*.

interchange has occurred at the positions indicated by the arrows in figure 1, *a* and *b*. Several figures were obtained in which these chromosomes could be followed for their entire lengths. Many more were found which were clear for the interchange region. The break in the *P-br* chromosome is about $^3/_4$ of the distance from the end of the longer arm, that in the satellite chromosome is somewhere between the second and end chromomeres of

the satellite itself. In all probability, only the terminal chromomere is involved in the interchange. It is certain that it includes no more than two chromomeres of the satellite. The two chromosomes resulting from the interchange are represented in figure 1, *c* and *d*. The synaptic configuration observed in plants heterozygous for the interchange is given in figure 2. The distinctive end chromomere on the normal satellite chromosome and its homolog on the interchange chromosome are represented as unsynapsed. Although this chromomere can be readily identified, no case of synapsis of these two homologous chromomeres was observed. Thus, although an interchange had occurred, the configuration at pachytene is practically a T.

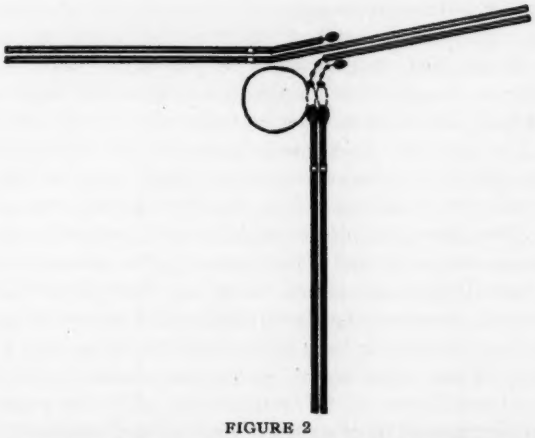

FIGURE 2

Diagram showing the pachytene configuration in a plant heterozygous for the interchange. The terminal knobs of the satellite fail to synapse.

The chain at diakinesis is presumably the result of this earlier lack of synapsis.

In the interchanges which involve large segments of chromosomes, the sterility approximates 50 per cent. The interchange here described shows but 25 per cent pollen sterility. The low sterility might be due to a distribution of the four chromosomes at anaphase I which decreased the proportion of abortive types. It should be possible to determine the type of distribution from the configurations at metaphase I. As shown above, synapsis to form a ring of 4 probably always fails at one point, namely the terminal chromomere of the satellite. Therefore the order of the chromosomes in the chain should be constant. This makes it unnecessary to recognize the four chromosomes to determine the type of distribution. If they

are numbered 1,2,3,4 starting at one end of the chain, 2-by-2 distribution might be 1 4-2 3 (adjacent), 1 3-2 4 (alternate) or 1 2-3 4 (adjacent). None of the latter type of adjacent distribution was observed. Of 379 metaphase I figures in which the type could be determined, 180 were alternate, 199 were adjacent. The alternate distribution gives rise to two nuclei, each possessing the full chromatin complement and should be fully fertile. The adjacent distribution gives rise to two nuclei, both of which are deficient, one for a long segment of the *P-br* chromosome, and the other for one or two chromomeres of the satellite. Since the alternate and adjacent distributions occur with about equal frequency, the sterility should be about 50 per cent if both types of deficiencies result in abortive spores. The type which is deficient for a part of the satellite might not abort, since it involves such a minute part of the monoploid complement. In that case, only 25 per cent sterility would be expected. Since this deficient type also carries a duplication for a large section of the longer arm of the *P-br* chromosome, it would be expected to function rarely, if at all, through the pollen (see page 1). Therefore in determining if this type of gamete functions, progeny of crosses with normal plants using the low sterile as the female parent were studied. From the observations on anaphase I distributions given above, the plants should be of three types: One showing 10 bivalents at diakinesis and giving normal pollen, one showing a chain similar to that of the female parent and giving about 25 per cent sterility, and one which is monosomic for the terminal one or two chromomeres of the satellite and trisomic for a long segment of the longer arm of the *P-br* chromosome. These three types should occur with equal frequency. The observed results were 9:19:1, respectively. The one plant which resulted from the functioning of a gamete with the small deficiency was readily distinguishable at diakinesis. Contrasted with the ordinary low sterile type in which chains are always formed, this plant forms chains and "pairs." "Pairs" were formed somewhat more frequently than chains, actual counts giving 213 "pairs" : 151 chains. The satellite "pair" is unequal, one member being much longer than the other due to the addition of the long piece of the *P-br* chromosome in place of the small terminal portion of the satellite. The other nine pairs are normal.

The pollen on this one plant having the small deficiency showed about 2 to 3 per cent sterility, the amount usually found in normal plants. It also appears to have a normal set of seed. This clearly demonstrates that the 25% sterility in the type which always forms chains is a result of non-abortion of the microspores deficient for the small piece of the satellite. It also shows that this type of gamete may survive and function in the ovules. The reason for the deviation from the expected 1:1:1 ratio is not clear. A possible explanation may be differential viability. Germination in this culture was only 66 per cent. Since this new type is trisomic for a part

of the *P-br* chromosome, it may be weaker than its sibs and be eliminated in a culture showing poor germination. The test is being repeated.

In the progeny from selfing a low sterile plant, the type homozygous for the interchange has been isolated. At diakinesis in this type there are ten pairs. The interchange chromosome attached to the nucleolus now has a long arm on either side of its point of attachment. The resulting chromosome is much longer than any of the other chromosomes. In prophases, the other interchange chromosome clearly shows that it is homozygous for the distinctive terminal knob of the satellite on the end of what is now the short arm.

Data on the inheritance of low sterility are too meager to draw any definite conclusion. The crosses with standard normal lines using the original low sterile plant as the pollen parent gave only a small proportion of plants showing low sterility. One plant from the second generation gave similar results in the cross of normal × low sterile, the total being 260 normal : 40 low sterile. In the third generation, several plants gave very close to a 1:1 ratio. In cultures studied cytologically, one gave 12 plants with 10 bivalents and 21 plants showing a chain of 4; another gave 10 : 6. In all cases, only the plants having chains showed sterility in the pollen. The original pollen classification was rechecked independently. No errors were found. A tentative explanation of the deviations from the theoretical 1 : 1 ratio is that a factor similar to that causing low sugary ratios (Emerson, 1925, Mangelsdorf and Jones, 1926) may have been present at some distance from the break in either of the two chromosomes. Crossovers would make it possible to isolate lines giving 1 : 1 ratios as well as those giving a high proportion of low steriles.

The fact that the gamete carrying the small deficiency may function furnishes a means of testing genes for their presence in this portion of the chromosome. The one plant which arose from a gamete of this type was from the cross of low sterile × albescent. According to cytological and genetical evidence reported by McClintock (1932), the order must be satellite-albescent-*Y-Pl*, *Y* and *Pl* being definitely located on the long arm. The plant obtained was not albescent, indicating that this factor is not located in the terminal chromomere of the satellite.

If this same deficient gamete which also carries a duplication for a part of the *P-br* chromosome does function in its theoretical frequency where germination is good, selfs of low sterile plants heterozygous for factors in the *P-br* chromosome should give trisomic ratios for those factors which lie in the arm involved in the interchange and disomic ratios for the remainder.

The writer's cytological observations on semisterile -3 in which the interchange also involves the longer arm of *P-br;* together with the genetic linkage observed there with brachytic indicate that the low sterile reported here will also show close linkage with brachytic. The tests are in progress.

Discussion.—In the semisteriles where critical cytological evidence has been obtained, there has been an interchange involving relatively large sections of the chromosomes (McClintock, 1930, Brink and Cooper, 1931, Rhoades, 1931). In these cases, a ring of four chromosomes is formed at diakinesis. Disjunction of the four chromosomes at anaphase is such that approximately 50 per cent of the spores are deficient for relatively large sections of the monoploid complement; thus accounting for the sterility in these types. The type heterozygous for the attachment of a fragment of one chromosome to a non-homolog, termed "simple translocation" in Drosophila, would be expected to give a T-shaped configuration at pachytene, a chain in place of a ring at diakinesis (see note at end), and only 25 per cent sterility. Brink and Cooper (1931) in an abstract have described a case assumed to be of this type. The observations presented in this paper demonstrate that a type which is definitely an interchange may exhibit all these features.

It is probable that some of the types suspected of being simple translocations in Drosophila would prove to be interchanges if critical cytological evidence could be obtained.

In the case reported here where one of the interchanged pieces is short, chain configurations are formed at diakinesis. If both pieces were very short, ten "pairs" might be formed regularly, giving no evidence that an interchange had occurred.

Note.—The author has pointed out (Burnham, 1930) that a "simple translocation" might give rise to rings in place of chains as a result of 4-strand crossing-over. This would occur only if the opening out at diakinesis were equational at the spindle fibre region. The case described here in which chains are always formed at diakinesis may be taken as evidence that the opening out is reductional at the spindle fibre region.

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A STRAIN OF MAIZE HOMOZYGOUS FOR SEGMENTAL INTERCHANGES INVOLVING BOTH ENDS OF THE P-BR CHROMOSOME[1,2]

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Semisterile-1 in maize involves an interchange of terminal segments between the *B-lg* and *P-br* chromosomes. The probable positions of the breaks in relation to several genes known to lie in the respective linkage groups concerned are depicted in figure 1.

The principal numerical data upon which the map is based are given in an earlier publication.[3] It has been established more recently that brown midrib (bm_2), which gives about 41 per cent crossing-over with semisterile-1, lies in the *P-br* chromosome some 40–50 units from *f* on the side

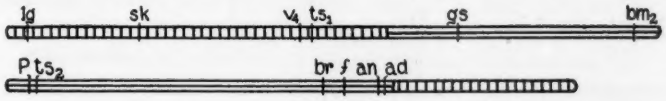

FIGURE 1

Chromosome map of the segmental interchange involved in semisterile-1. The exact positions of the ts_1, ts_2, *an* and *ad* are not yet worked out although they are known to be in the regions indicated.

remote from *P*.[4] Green striped (*gs*) gives about six per cent recombination with semisterile-1 in back-crosses and about 27 per cent with bm_2. While the confirmatory tests are not yet completed, the available evidence indicates that both *gs* and bm_2 are on the translocated piece of the *P-br* chromosome.

Some doubt has arisen in regard to the validity of the respective percentages of crossing-over earlier reported for *an*, *ad* and semisterile-1.[3] The two supposedly semisterile *ad* segregates found have proved to breed as normals, as have three plants which were originally classified as semisterile *an*. It would appear that some extraneous factor was responsible for the aborted pollen found in these plants, and that crossing-over between the two respective genes and the point of break either does not occur at all or is substantially less than earlier given.

As Burnham[5] first reported, semisterile-1 plants show eight bivalents and a ring of four chromosomes at diakinesis. Semisterile-5 exhibits a similar configuration, the ring being attached, however, to the nucleole.[6] Semisteriles-1 and -5 in combination form a ring of six chromosomes and seven bivalents (Fig. 2). They consequently involve one chromosome in common.

FIGURE 2

Ring of six chromosomes and seven bivalents in hybrids between semisteriles-1 and -5.

Linkage tests show that the chromosome common to the two semisteriles corresponds to the *P-br* group. Semisterile-5 and ts_2 give 24.0 per cent crossing-over. This value is based on counts of 254 Ts_2 plants in three back-cross progenies which showed 32.0, 9.75 and 27.3 per cent recombination, respectively. The position of the point of break in relation to *br* and *f* may be inferred from the data from a single progeny presented in table 1. The amounts of crossing-over shown are as follows: semisterile-5 and *f*, 16.0 per cent; semisterile-5 and *br*, 13.4 per cent; *br* and *f*, 2.7 per cent. A back-cross progeny of 100 plants segregating semisterile-5 and bm_2 gave 34.0 per cent recombination.

TABLE 1

DISTRIBUTION OF PLANTS FROM THE BACK-CROSS SEMISTERILE-5, *Br F. br f* x NORMAL, *br f*

SEMISTERILE				NORMAL				TOTAL
Br F	*Br f*	*br F*	*br f*	*Br F*	*Br f*	*br F*	*br f*	
32	1	0	4	6	0	1	31	75

The other chromosome involved in the semisterile-5 ring corresponds to the *Y-Pl* linkage group. The summarized data from six small progenies bearing on this relationship are presented in table 2.

TABLE 2

DISTRIBUTION OF PLANTS FROM BACKCROSSES OF THE TYPE, SEMISTERILE-5 *Y pl.-y Pl* x NORMAL, *y pl*

SEMISTERILE				NORMAL				TOTAL
Y Pl	*Y pl*	*y Pl*	*y pl*	*Y Pl*	*Y pl*	*y Pl*	*y pl*	
0	30	3	20	18	4	22	1	98

The amounts of crossing-over indicated are as follows: semisterile-5 and *Y*, 45.9 per cent; semisterile-5 and *Pl*, 8.2 per cent; *Y* and *Pl*, 39.8 per cent.

On the basis of the linkage data presented in summary form above the semisterile-5 segmental interchange may be mapped as in figure 3.

The *Y-Pl* chromosome is attached by its satellite end to the nucleole. The cytological evidence shows that it is the opposite end of this chromosome which is involved in the semisterile-5 segmental interchange. This

fact together with the linkage data bearing on the relation of *Y* and *Pl* to the point of break demonstrates that the order in the chromosome is nucleole-*Y*-*Pl*-break. A revision is necessary of our earlier report[6] regard-

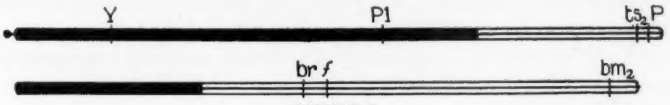

FIGURE 3

Chromosome map of the segmental interchange involved in semisterile-5.

ing the relative lengths of the chromosome segments involved in the semisterile-5 interchange. The error was one of statement rather than of observation, and we are indebted to Miss McClintock for calling it to our attention. In semisterile-5, according to our measurements of early prophase figures in pollen mother cells, approximately one-third of the satellite chromosome (*Y*-*Pl*) is interchanged with about two-thirds of the *P*-*br* chromosome. These relations are depicted in figure 4 which is intended to replace the corresponding figure in our earlier publication.[6]

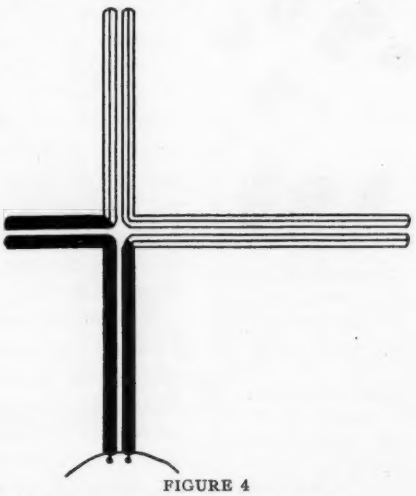

FIGURE 4

A diagrammatic representation of the cross-shaped figure found at early prophase in semisterile-5. The chromosome shown entirely in black and attached to the nucleole corresponds to the *Y*-*Pl* linkage group.

When semisteriles-1 and -5 are crossed, three kinds of offspring result, namely, normals with all sound pollen, semisteriles with 50 per cent of the pollen aborted and a third class in which only 15–25 per cent of the pollen is functional. The latter group of individuals is heterozygous for the two segmental interchanges and shows seven bivalents and a ring of six chromosomes at diakinesis (Fig. 2). Among the normal offspring of such double semisteriles selfed four classes of plants differing in the structure of their chromosomes are expected: (1) the standard type, which is designated *o*-normal; (2) two classes of *x*-normals, *x*-normal-1 and *x*-normal-5, homozygous, respectively, for the segmental interchanges characteristic of semisteriles-1 and -5; and (3) the double *x*-normal form (*x*-normal-1.5) homozygous for both segmental in-

terchanges. These three groups should be distinguishable on the basis of their reactions in crosses to the *o*-normal or standard type. In such matings *o*-normals should give only normal offspring, the two kinds of *x*-normals should give semisteriles, and the double *x*-normal should yield plants with, presumably, a much lower percentage of functional pollen.

A test of this type has been completed on two fully fertile segregates from a self-pollinated hybrid between semisteriles-1 and -5 which produced 85 per cent aborted pollen. One of the plants in question was an *o*-normal, since, in crosses with the standard line, its offspring were fully fertile. The other plant proved to be a double *x*-normal (*x*-normal-1.5), giving hybrids with the standard line in which upward of three-quarters of the pollen grains were empty and shriveled. The offspring of the two fertile segregates selfed were all fully fertile.

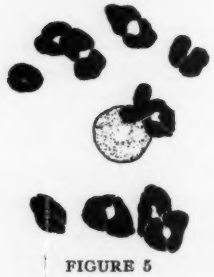

FIGURE 5

Diakinesis in a pollen mother cell of *x*-normal-1.5. Ten bivalent chromosomes are present in this type which is homozygous for two segmental interchanges involving opposite ends of the *P-br* chromosome.

Cytological examination of one plant in the progeny adjudged to be *x*-normal-1.5 revealed ten bivalent chromosomes as expected (Fig. 5). While forming ten pairs, such plants differ from standard maize in that the *P-br* chromosome is replaced by a composite one whose interstitial segment consists of the middle part of the *P-br* chromosome but whose respective ends correspond to parts of the normal *B-lg* and *Y-Pl* chromosomes, the latter being altered in a complementary way.

Hybrids between the *x*-normal-1.5 line and the *o*-normal type show a six-armed chromosome complex in the pollen mother cells at early prophase. Figure 6 is based on a preparation in which the relations of the parts in the interchanged chromosomes were particularly clear. The interchange group (shown in black) is attached to the nucleole by the satellite end of the *Y-Pl* chromosome. Two well-defined chiasmata are in evidence from each of which three arms extend. The upper chiasma in the figure corresponds to that in semisterile-5 which involves an interchange between the *Pl* end of the *Y-Pl* chromosome and the *P* end of the *P-br* chromosome. The lower chiasma is that characteristic of semisterile-1 in which the v_4 end of the *B-lg* chromosome is exchanged with the opposite (bm_2) end of the *P-br* chromosome. Between the two chiasmata lies an interstitial segment of the *P-br* chromosome corresponding to a map distance of roughly 25 units. The loci of the *br* and *f* genes are in this piece.

The plant from which the homozygous *x*-normal-1.5 stock was derived resulted from a cross between semisteriles-1 and -5. In this individual the two segmental interchanges were present consequently in repulsion phase.

In order that *x*-normal-1.5 gametes be formed by such a plant crossing-over must occur in the region between the semisterile-1 break in one interchanged *P-br* chromosome and the semisterile-5 break in the other interchanged *P-br* chromosome. Such a crossing-over, followed by distribution of alternate chromosomes in the ring to the same pole (and non-disjunction if crossing-over occurs in the four-stand stage), would give rise to *x*-normal-1.5 and *o*-normal nuclei (Fig. 7). It is a rather remarkable circumstance that the two fertile derivatives tested proved to be of these two particular

FIGURE 6

Early prophase stage in a hybrid between standard maize and the doubly interchanged type, *x*-normal-1.5 showing a six-armed complex and seven bivalent chromosomes.

complementary types; both must have resulted from combination between like crossover gametes of the two respective kinds.

As may be seen from figure 6, pairing of the chromosomes in the semisterile-1.5 complex is almost complete. In each of the six arms and the interstitial segment, corresponding parts lie in close association. In this case, therefore, as in semisteriles-1 and -5 considered earlier, there is definitive evidence that translocation in itself does not radically alter the ability of the chromosomes concerned to pair. Only at the chiasmata where the strands change partners does the relationship appear to become somewhat

looser. On the basis of the cytological evidence alone one might well doubt with reference to these cases in maize whether the effect of segmental interchange on effective pairing extends beyond the chromosome parts immediately adjacent to the chiasmata. Data on crossing-over in these re-

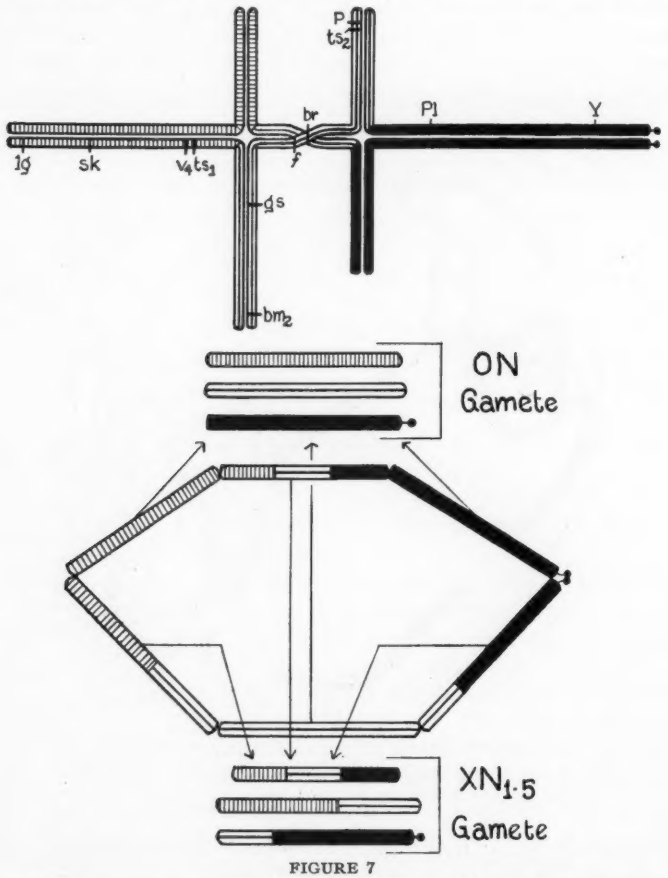

FIGURE 7

Diagrammatic representation of the origin of the *x*-normal-1.5 type through crossing-over in the interstitial segment of the ring of six chromosomes found in hybrids between semisteriles-1 and -5.

gions are needed. Until there is experimental evidence to support it Darlington's[7] *a priori* argument that the amount of pairing of corresponding parts of two chromosomes is lessened when the homology is not continued in adjoining segments is not to be taken too seriously.

The estimated length of the interstitial segment in the doubly interchanged *P-br* chromosome, as stated above, is about 25 crossover units. It is not yet definitely known what the effect on amount of crossing-over is of the two structural changes in the region proximal to the two breaks. That crossing-over must occur rather freely in this segment, however, is indicated by the fact that of the two normal segregates from a semisterile-1.5 plant (repulsion phase) selfed both were crossover types. It would appear that in this case, therefore, crossing-over between corresponding parts is not confined to the arms of the interchanged chromosome complex.

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[2] The authors desire to express their appreciation for the assistance received from the Research Fund of the University of Wisconsin, the National Research Council and the Elizabeth Thompson Science Fund.

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CHROMOSOME RINGS IN MAIZE AND OENOTHERA[1,2]

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As Håkannson,[3] Darlington,[4] Cleland and Blakeslee,[5] and Emerson and Sturtevant[7] have shown, Belling's[8] hypothesis of segmental interchange affords a consistent explanation of the chromosome attachments in Oenothera. It is by no means clear, however, to what extent the other peculiarities in cytological and breeding behavior of this genus are thus accounted for. Renner's[9] extensive researches on the genetic interrelations of the Oenotheras demonstrate that not only do certain genes display anomalous linkages but also many characters which segregate freely in some crosses assort in stable groups in others. In the origin and integrity of the "complexes" of Renner lie the main problems which the Oenotheras present. Cleland[10] has established the fact that ring-formation is a characteristic feature of certain Oenothera species and, furthermore, that the chromosomes within the rings are distributed, for the most part, in a determinate

way at the heterotypic division. These important observations constitute the point of departure in the more recent studies.

Chromosome rings appear to have been incorporated as an integral part of the hereditary mechanism in several wild species of Oenothera. How far one may go in arguing the problems which this genus presents from a plant like maize in which rings have recently been discovered and in which this structural peculiarity of the germplasm is maintained in the stock only by controlled breeding and cannot in any sense be considered characteristic of the species, is perhaps a debatable question. Interchange of terminal segments between non-homologous chromosomes, however, has been postulated as primarily responsible for the main features of Oenothera behavior. Since several structural changes of this type have now been studied in maize it is pertinent to inquire in how far they lead in this species to the same consequences as have been attributed to them in Oenothera.

One is impressed at once with the non-conformity between Oenothera and maize rings with respect to the manner in which the constituent chromosomes assort on the heterotypic spindle. According to Cleland[11] adjacent chromosomes pass to opposite poles in 80 per cent of the P.M.C. of *Oe. Lamarckiana*. In other ring-forming members of the genus the behavior is similar. In maize, on the other hand, as McClintock[12] has shown for the ring of four in semisterile-2, and as our own studies[13,14] reveal in semisteriles-1 and -5 with their rings of four, the chromosomes in the interchange complexes are distributed two by two at random. In semisterile-1.5, which involves a ring of six, there is likewise no regular tendency for the chromosomes to become arranged on the spindle in zig-zag fashion, even though they remain attached to each other at this stage. The functional pollen grains must result from this type of distribution, but they constitute not more than 20–25 per cent of all the microspores formed. Recent cytological observations on hybrids between x-normal-3 and x-normal-1.5, which form rings of eight and are about 75–80 per cent sterile, indicate that here too the chromosomes are distributed indeterminately at the first meiotic division. While all the rings thus far studied in maize are relatively small, it is important to note that as they increase in size there is no marked tendency for the chromosomes to arrange themselves in zig-zag order on the heterotypic spindle. It is apparent, therefore, that in maize simple segmental interchange does not lead to the determinate type of chromosome assortment observed in Oenothera.

The necessary condition of stability of a Renner complex is that when two complexes are opposed to each other in a hybrid there shall be no interchange of materials between them. Recombination of the hereditary materials which distinguish the complexes only rarely occurs. It is not easy to understand how this degree of stability is maintained if there is nothing more involved than simple reciprocal translocation of terminal segments.

In semisterile-1 maize, which involves a segmental interchange between the *B-lg* and *P-br* groups, it is found that crossing-over takes place as freely in the two opposite arms of the ring tested as it does in these chromosome parts in normal plants.[13] Rhoades[15] has shown that the crossover values observed in the semisterile-4 ring (*B-lg* and *Pr-v*$_2$ linkage groups) are of the same order of magnitude as those in the standard map. In the semisterile-5 ring which resulted from an interchange between the *Y-Pl* and *P-br* chromosomes the evidence at hand indicates that the frequency of crossing-over is significantly reduced in both arms of the *P-br* chromosome but not in *Y-Pl* (see preceding paper). The observed decrease in the *P-br* group, however, is only about one-half, so that the residual amount of crossing-over is still large enough to permit of ready interchange of genes between the opposed chromosomes. The linkage relations in the interstitial segment of the *P-br* chromosome in semisterile-1.5 have not yet been studied. But the fact that the double *x*-normal type (*x*-normal-1.5) was obtained in one of two normal plants tested among the progeny of a selfed hybrid between semisteriles-1 and -5 demonstrates that crossing-over in this region is not blocked. The cytological figures for semisteriles-1, -5 and -1.5, moreover, reveal that the chromosomes in these rings establish a close synaptic relation in meiotic prophase.

The present evidence from maize throws no light on the question whether in the immediate vicinities of the points of break and reattachment the frequency of crossing-over in the rings is altered. With a reservation then with respect to these localities, it is clear that segmental interchange in maize has not led to the establishment of a basis for complexes such as those in Oenothera.

Darlington[16] has recognized this limitation of the Belling hypothesis and, in seeking to meet it, assumes that the chromosomes in opposing complexes in Oenothera are differentiated in the regions proximal to the pairing ends. Logically, this idea is sound, but one is left in some doubt as to the nature of the interstitial segments which Darlington postulates when he says (page 430), "The middle regions of the chromosomes forming one complex consist of an arrangement of chromatin unrelated with the opposite complex, but probably related genetically in that the same materials are present but in different proportions."

While the rather wide variations in physiological properties as manifested in both gametophyte and sporophyte might suggest differences in gene balance, it is sufficient for the purposes of our ensuing discussion to adopt the simpler view that the various Renner complexes differ in genic composition with respect to certain allelomorphs. The high degree of stability of the complexes can only mean that in hybrids crossing-over between complexes rarely or never occurs. As the results in maize show, segmental interchange alone does not explain this fact. The integrity of

the complexes can be accounted for, however, if it is assumed that the segments containing the differential genes lie in reverse order when two complexes are opposed.

In an extended analysis of certain races of *Drosophila melanogaster* in which crossing-over is markedly reduced in one or the other limb of the long chromosomes, Sturtevant[17] has furnished the proof of his earlier suggestion that the behavior is due to the inversion of chromosome segments. This conception when applied in conjunction with the segmental inter-

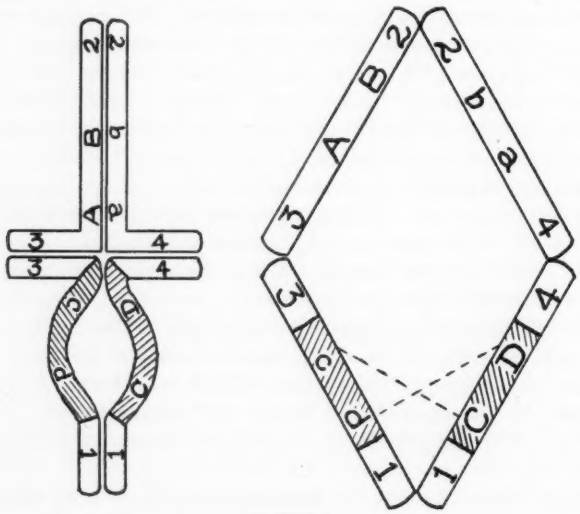

FIGURE 1

Schematic representation of the origin of a chromosome ring with a relatively reversed interstitial segment. At the left is shown the assumed relations between the chromsome parts during synapsis. At this stage the reversed segments, which are cross-hatched, fail to pair. At the right, the ring which arises through the opening out of the synaptic complex until the ends only remain attached, is illustrated.

change hypothesis appears to account satisfactorily for the stability of the complexes in *Oenothera* and helps to explain the peculiar linkage relations of certain of the genes. The essential differences in the properties of the chromosome rings in maize and Oenothera may be due to the circumstance that in the latter group segmental interchange has been accompanied by inversion while in the former it has not.

The simple manner in which reciprocal translocation of terminal segments may produce inversions directly can best be illustrated by a hypo-

thetical example. Let two non-homologous chromosomes in a regularly pairing species be represented as *1-a-b-2* and *3-c-d-4*. The numbers denote end segments of the chromosomes and the letters, loci proximal to them. Suppose end *1* of the first chromosome becomes interchanged with end *3* of the second and that a race homozygous for the alteration is established. Assume further that another race arises similarly in which end *1* of the first chromosome is interchanged with end *4* of the second. That is, a given end of one chromosome is interchanged with opposite ends of a second chromosome. If these two races now cross a ring of four chromosomes will result in which the two *c-d* segments are reversed with respect to each other as in figure 1. If the breaks do not occur at the same level in both inter-

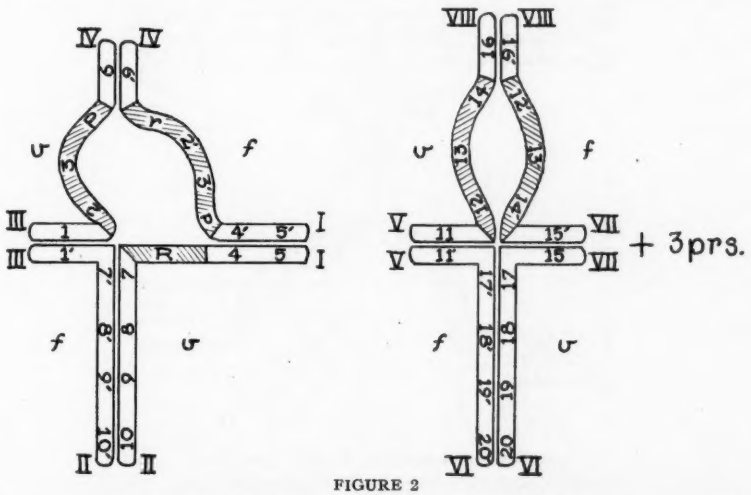

FIGURE 2

The assumed positions of the *R* and *P* genes in relation to the inverted interstitial segments comprising the bases of the *velans.flavens* complexes.

changes blocks of genes in two chromosomes will be inverted with respect to the corresponding factors in a third. The process of interchange may be continued to give larger rings in which the middle portions, with the spindle fibre attachments, in all but one pair of chromosomes are reversed. The mechanical difficulties incident to pairing in such forms would be expected strongly to depress crossing-over, as in fact Sturtevant[17] has shown to be the case with inverted segments of Drosophila. If the Oenothera complexes have their bases in segments which are thus reversed when in opposition the genic differences between them will be maintained.

Whether reversal of interstitial segments influences orientation of the chromosomes in the rings on the heterotypic spindle cannot be said. One

can only surmise that some such mechanism must account for the markedly different behavior in this respect of maize and Oenothera.

In addition to providing an explanation of the stability of the complexes the hypothesis of inverted interstitial segments assists in accounting for the peculiar way in which certain genes are transmitted in relation to each other and to the complexes with which they are associated. For purposes of illustration the breeding facts which Renner[4] has obtained relative to *P* (red papillae) and *R* (red midrib) may be considered. In the present state

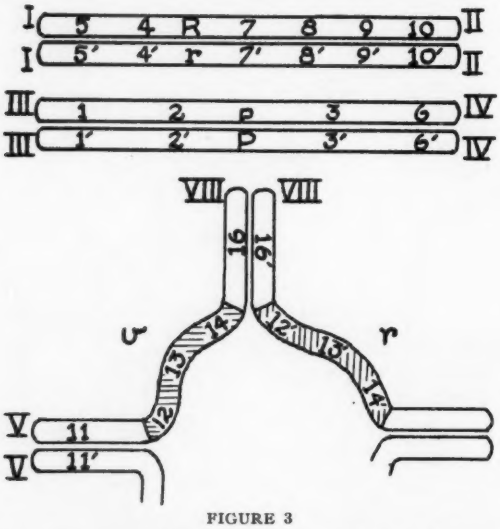

FIGURE 3

The assumed positions of the *R* and *P* genes in independent chromosome pairs when *velans.* is opposed to *rigens.* The complex remainders are assumed to have their bases in inverted chromosome segments in the ring.

of development of the Oenothera researches the explanation offered must necessarily be of a formal character.

In *velans. flavens* which, as Cleland and Oehlkers[18] have shown, has two rings of four and three pairs, *P* and *R* are linked to each other but are independent of the remainders of the respective complexes. Following Cleland and Blakeslee[6] and Emerson and Sturtevant[7] it may be assumed that the loci of the two genes are in the same ring (Fig. 2). Arabic numerals (primed in the chromosomes of one complex) are used to designate a sufficient number of loci to show the relative positions of homologous parts. It will be noted that both *r* and *p* are assumed to be in one chromosome while their respective allelomorphs occupy positions one in each of the re-

versed segments of the two adjacent chromosomes. In the second ring the segment 12–14 in a *velans.* chromosome is reversed with respect to 12′–14′ in the adjacent *flavens* chromosome. It is assumed that these reversed parts also carry genes in which the complexes differ and that the inversion prevents crossing-over between them. The chromosomes in this ring assort independently of those in the first.

In *rigens. curvans*, which has a ring of 14 chromosomes, *P* and *R* are linked and both are linked with the remainders of these complexes. These

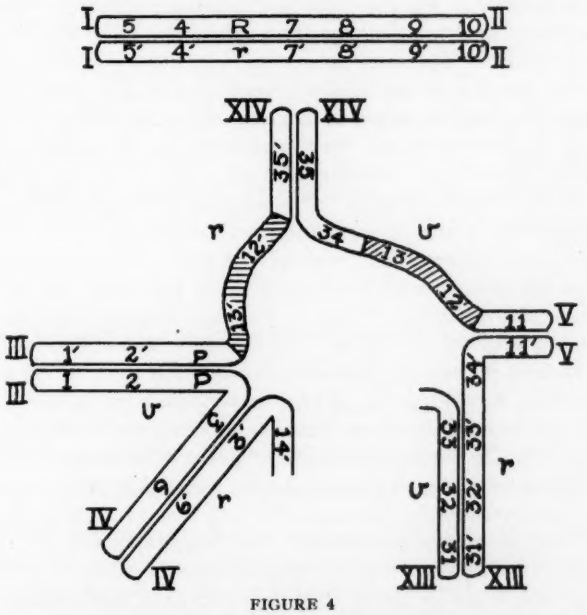

FIGURE 4

The assumed relations between *R* and *P* in *velans. rubens.* *R* is located in an independent pair of chromosomes while the *P* locus is in the ring adjacent (in one chromosome) to an inverted segment.

facts are satisfied if it is assumed that the genes in question lie in relatively inverted segments as in figure 2, all 14 chromosomes, however, being brought into a ring through interchange of ends. Since *rigens. curvans* breeds true for the ring of 14 chromosomes it must be further postulated that crossing-over is prevented in the interstitial parts of the chromosomes not containing *P* and *R* by reversal of differential segments, or that the crossover types of spores are inviable on account of genic unbalance in them.

When *rigens.* and *velans.* are opposed, *P* and *R* are independent of each

other and both are independent of the remainders of the complexes. We may assume as Cleland and Blakeslee[6] have done that these genes now lie in different pairing chromosomes (Fig. 3). The remainders of the complexes presumably have their bases in relatively inverted interstitial segments in the ring. Even with crossing-over in the ends, distribution of alternate chromosomes to the same pole will then maintain the integrity of the complex remainders.

In *velans. rubens P* and *R* are independent of each other but *P* is linked with the remainder of the complex. Occasionally, however, *P* may cross over from *velans.* to *rubens.* It may be assumed that *R* lies in an independent pair of chromosomes. As illustrated in figure 4, *p* may be presumed to occupy a position in one of the pairing ends of the *III-XIV rubens* chromosome but directly adjacent to a segment which is reversed with respect to its homolog in *velans.* In this relation *P* will usually assort with *velans.* and *p* with *rubens.* Crossing-over may occur at rare intervals, however, in the short region between the *p* locus and the inverted segment thus transposing *P* and *p* with respect to the two complexes.

Another circumstance which may play a part in the maintenance of stability of the complexes in Oenothera hybrids with relatively large rings is the inviability of certain types of crossover spores. The point may be illustrated with the semisterile-1.3.5 case in maize. Semisteriles-1, -3 and -5, respectively, involve translocations between the *P-br* chromosome and three other chromosomes. When two of these interchanges (1 and 5) were obtained together in homozygous condition the stock was crossed with *x*-normal-3. In the ring of eight chromosomes in the resulting hybrid there are two regions in which single crossing-over, followed by the distribution of alternate chromosomes to the same pole, will lead to nuclei deficient for some part of the nuclear complement, and hence abortive. If crossing-over in one of the regions, however, is accompanied by crossing-over in the other, two new types of spores will result, *o*-normal and *x*-normal-1.3.5, both of which would be functional. Such simultaneous double crossing-over is not to be expected often if the segments in question are short. In larger rings of similar nature the chances of simultaneous compensatory crossing-over would become very small indeed. Thus without relative inversion of interstitial segments certain blocks of genes entering a hybrid of this sort would usually segregate out intact as in Oenothera. It is to be noted, however, that the arrangement described is not operative in rings containing less than eight chromosomes. Consequently, it can only be considered as ancillary to some more fundamental mechanism which in the Oenotheras operates to restrict crossing-over in smaller rings as well.

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ON THE STABILITY OF PHYSIOLOGICAL CHARACTERS OF BACTERIA

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Read before the Academy, Monday, April 25, 1932

Variation as a broad biological principle is well recognized today and it may fairly be questioned how far-reaching are the possibilities for its operation within the unique and complex community of the bacterial culture. The enormous number of cell generations within a short period of time might suggest the possibility of rapid changes in cultures of microorganisms. How far does the old query concerning the leopard changing his spots apply to the bacterial culture under ordinary laboratory conditions? What is the net effect of long cultivation in artificial media of fairly uniform composition upon a given bacterial culture? These are questions we may well ask and attempt to answer in these days of frequent reports on the "changing bacteria."

Variation, in the sense of gain or loss of demonstrable characters by microörganisms cultured under the usual laboratory conditions, is in fact a broad term as has been shown by Jordan, 1915, and others. It is well known that bacteria exhibit temporary fluctuations from transfer to transfer. These variations are dependent upon inherent adaptability of the

organisms to small and uncontrollable factors in the environment and are in the main offset in the "average" behavior of the culture. Temporary changes may also be due to the development of a latent characteristic, called forth by some new element in the environment. Such variations may be expected to persist as long as the particular cultural environment permits. Good examples of this type of change in a culture were cited by Jordan in 1915.

Other variations, which are more or less permanent in cultures, are conceivably of two types: those concerned in possible life cycles or dissociative changes in the bacteria, and those of a deteriorative nature, resulting from prolonged exposure to the so-called "artificial" conditions of the laboratory culture. It is the purpose of the present paper to consider the latter type, citing cases of observations on certain biochemical characters of cultures freshly isolated and of the descendants of those cultures after some years of existence in the laboratory collection of stock cultures. The need for such a presentation of facts appears in the general impression expressed in the following statement from a recent text book of bacteriology: "Pure cultures of bacteria and other microörganisms are often weakened when grown on artificial culture media, or may lose altogether their capacity of producing the change which they carry on in nature, as in the case of *Azotobacter* and other nitrogen-fixing bacteria." If this be true it would appear to be a cogent argument in support of the claim that variation in activity of pure cultures of bacteria under laboratory conditions is such that there is little value in physiological reactions for separation of species.

The author does not wish to imply that bacteria are unchanging in the face of any environmental conditions, for it has been known since the days of Pasteur, 1881, that environmental stimuli may induce bacterial variations. No one questions the fact that bacterial variation is a common occurrence. However, there appears to have developed an exaggerated idea of the magnitude of these changes. It is the experience of many bacteriologists who have observed stock cultures of particular species of bacteria over a period of years that under properly controlled conditions these organisms can be maintained with but little change in biochemical characters. This tendency toward uniformity of the various cultures used in preparation of bacterial products is likely to be forgotten in the glare of attention which is now focused on the problem of bacterial variation. Striking examples of such stability, covering the activities of a wide variety of bacteria, spore-forming and non-spore-forming, motile and non-motile, aerobic and anaerobic, are therefore cited here.

Nitrogen-Fixing Bacteria.—The facts are available concerning the three great divisions of the nitrogen-fixing organisms—the symbiotic rhizobia or root nodule organisms of the Leguminosae, the aerobic *Azotobacter* and the anaerobic *Clostridium*.

One particular strain of rhizobia from the nodules of alfalfa has been carried in pure culture in this laboratory since 1912. It was originally obtained from a well-isolated colony on a Petri plate and has been carried in our stock culture collection on slopes of mannitol agar with and without the presence of combined nitrogen. Incubation has been at room temperature with transfers at intervals of 60 to 90 days. With the exception of 4 of the early years, 1913, 1914, 1916 and 1917, this culture has been used under controlled conditions in tests of cultural characters and also in tests of nodule production. A brief summary of the results of these tests follows:

RHIZOBIUM MELILOTI FROM ALFALFA, 1911

1912 pure, cultural characters typical, nodules formed
1913 no test
1914 no test
1915 pure, cultural characters typical, nodules formed
1916 no test
1917 no test
1918 to 1931 pure, cultural characters typical, nodules formed

Numerous tests were made on these cultures during this time, but there was no indication of well-defined changes in cultural characters nor in ability to produce effective nitrogen-fixing nodules on alfalfa plants. Since 1927 this culture has been used for both greenhouse and field experiments, at the end of which the culture was isolated and compared with the original parent stock. No clean-cut change in characters was noted. It is significant that during the 20 years of almost unbroken study, this culture, *Rh. meliloti* 100, has thus remained so remarkably stable in respect to cultural characters and ability to form effective nitrogen-fixing nodules on alfalfa.

Somewhat similar examples might be taken from studies of other species of the nodule bacteria. Two strains of rhizobia of the soybean group, Cultures 503 and 504, are typical. Since 1921 these cultures have been tested each year in relation to their effect on the host. Culture 503 always benefits the growth of the soybean plant while Culture 504 exerts little if any effect on the host. Both cultures produce nodules.

For the non-symbiotic group, *Azotobacter*, the proof of stability is not so extensive, since it depends upon maintenance of only two characters; ability to fix free nitrogen and dark brown pigment production. In 1914 a pure culture of *Azotobacter* was isolated from garden soil at the University of Wisconsin. According to the general characters this culture belongs to the species *Azotobacter chroococcum* Beijerinck. It also has been carried in stock on mannitol agar. In 1916, 25 single cells were picked by means of the Barber apparatus and one of these which showed vigorous growth was selected for future study. At irregular intervals thereafter the de-

scendants of this single cell culture have been seeded on to mannitol agar, 100 cc. in 750-cc. Erlenmeyer flasks, and these incubated for 21 days. At the end of this period the entire contents of the flasks were analyzed for total nitrogen by the Kjeldahl method. The average amount of nitrogen fixed, calculated from 9 parallel flasks, minus the blank on the agar and chemicals, is shown below:

AZOTOBACTER (Single Cell Culture)

	GAIN IN NITROGEN MILLIGRAMS
1917	7.8
1918	6.9
1919	7.0
1920	7.0
1932	9.2*

* Last analysis carried out by Lundin and Ellburg method which usually gives higher results.

Thus for 15 years this single cell culture of *Azotobacter chroococcum* has retained with remarkable constancy its power to fix atmospheric nitrogen. In stock culture it still produces the dark brown pigment so characteristic of this organism. So far as the anaerobic nitrogen-fixing bacteria are concerned the results of Winogradsky, 1895, and Omeliansky, 1915, are pertinent. These investigators studied the assimilation of nitrogen by the same strain of *Clostridium pasteurianum* in the presence of glucose.

CLOSTRIDIUM PASTEURIANUM FROM WINOGRADSKY

	GAIN IN NITROGEN
1895	1.5 to 1.8 mg. per gram of sugar consumed
1915	1.4 to 1.7 mg. per gram of sugar consumed

Unlike the previous tests, the culture of *Cl. pasteurianum* was kept in the spore state for 20 years between tests (incidentally a spore culture might be expected by reason of its dormancy to retain its properties more certainly than would a non-spore-forming culture). As shown from the results of Omeliansky, 20-year-old spores did in a remarkable degree maintain that fundamental character of their species, namely, their ability to fix nitrogen. In this connection, the results of Van der Lek, 1930, are also of interest. From dry spores of *Clostridium* (*Granulobacter*) *butylicum*, held for approximately 40 years in the laboratory of Beijerinck, Van der Lek secured a culture which produced a vigorous fermentation similar to that of the original description of Beijerinck in 1893.

Lactic Acid Bacteria.—Another striking example of bacterial stability is displayed by *Lactobacillus pentoaceticus* which has been studied in our laboratories during a period of 13 years. Titratable acid from various sugars was measured after 1, 2, 4, 5, 11, 12 and 13 years. The general results were as follows:

EFFECT OF LONG TIME CULTIVATION ON THE PRODUCTION OF TITRATABLE ACID BY CULTURES OF LACTOBACILLUS PENTOACETICUS

CARBON COMPOUND	cc. 0.1 *N* ACID IN 10 cc. OF CULTURE — YEAR 1920 cc.	1921 cc.	1926 cc.	1927 cc.	1930 cc.	1931 cc.	1932 cc.
Arabinose	11.1	12.4	12.4	10.0	9.2	9.7	11.4
Xylose	10.4	9.4	11.4	11.8	9.4	...	11.3
Glucose	7.0	7.0	5.2	6.6	6.1	5.2	5.7
Galactose	5.4	5.3	...	4.4	5.3	4.8	5.1
Sucrose	4.2	4.2	5.9	5.3	5.5	3.7	4.5
Lactose	0.8	0.8	1.6	2.4	...	...	1.2
Raffinose	0.5	0.5	1.1	1.0	0.9	0.9	1.0
Melezitose	0.8	0.8	0.9	0.9	0.9	0.8	0.9
Mannitol	1.5	1.5	1.7	1.8	1.0	1.1	1.8
Salicin	0.5	0.5	1.8	1.7	2.2	1.6	2.1
Alpha Methyl Glucoside	4.7	4.7	5.1	4.6	4.7	...	5.1

These figures, although somewhat variable, indicate that this typical culture of the lactic acid bacteria from plants has not changed significantly in its fermentation characters. Transfers once every month on yeast-water agar have kept this culture in an active state and without appreciable loss of vigor in fermentation characters.

Growth of Tubercle Bacteria.—One of the most accurate methods of measuring bacterial growth is to remove the cells and weigh the dry bacterial substance. This procedure has been followed for 14 years with a culture of *M. tuberculosis* grown on a beef-infusion peptone, glycerol phosphate medium for the production of tuberculin. All the conditions such as temperature, reaction, time of incubation, etc., have been kept as uniform as possible. The dry-weights of the bacterial cells in grams per liter of the medium are shown. The table also shows the number of liters of medium used in each test.

COMPARISON OF THE WEIGHT OF GROWTH OF MYCOBACTERIUM TUBERCULOSIS

YEAR	AMOUNT OF CULTURE — LITERS	DRY WEIGHT OF CELLS PER LITER — GRAMS
1917–18	337	6.5
1918–19	569	7.2
1919–20	892	6.5
1920–21	731	7.2
1921–22	966	6.5
1922–23	641	7.0
1923–24	882	7.2
1924–25	626	7.0
1925–26	990	6.8
1926–27	1250	6.4
1927–28	998	6.2
1928–29	992	6.5
1929–30	1228	6.0
1930–31	596	5.8

The results of these large scale experiments show clearly that under uniform conditions the tubercle organism may be cultured for at least 14 years without deterioration in vigor of growth as evidenced by weight of the bacterial cells per unit of culture.

In this paper no attempt has been made to deal with bacterial dissociation or life cycles but rather to present certain observed facts which relate to the stability of different groups of bacteria when kept for years under suitable artificial conditions. To the author it appears that the supposition of gradual deterioration is not necessarily supported by facts. The results of the experiments here reported show that these forms may be kept in the laboratory for years and yet remain stable with respect to the properties tested. It is indeed significant that no matter how contrastive the artificial medium as compared with the natural environment to which the organisms were accustomed, many of the dominant physiological characteristics remain unchanged. The results of this study, therefore, support the fundamental concept of the stability of pure cultures of bacteria under suitable artificial conditions.

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*CONCERNING SEQUENCES OF HOMEOMORPHISMS**

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1. *Introduction.*—In his thesis, Knaster[1] defines a method of construction which he calls the method of bands, by means of which he is able to construct indecomposable continua of various types. These continua may also be thought of as examples to show that each member of a sequence of continua, each of which contains the following continuum of the sequence, may be homeomorphic with a rectangle, while the set of points common to all the continua of the sequence is not homeomorphic with a rectangle. The present paper is a contribution to the solution of the problem of determining conditions under which a homeomorphism

exists between the sets common to two sequences of point sets, the corresponding elements of which are homeomorphic. A certain principle of uniformity is used in this connection.

2. *Notation and Definitions.*—In the following, $[M_i]$ denotes a sequence of point sets: $M_1, M_2, M_3, \ldots$. For each i, there exists a homeomorphic transformation T_i of M_i, and we shall frequently denote $T_i(M_i)$ by N_i.

By saying that the sequence of points $[P_i]$ converges to the point P, we shall mean that the point P is the sequential limiting point of the sequence $[P_i]$.

A sequence of point sets $[T_i(M_i)]$ is said to be *uniformly homeomorphic* with respect to the sequence $[M_i]$, if given any positive number ϵ, there exist corresponding positive numbers δ and k, such that if $i > k$ and the distance between the points p and q of M_i is less than δ, then the distance between $T_i(p)$ and $T_i(q)$ is less than ϵ.

If the sequence $[M_i]$ is uniformly homeomorphic with respect to $[T_i(M_i)]$, and the sequence $[T_i(M_i)]$ is uniformly homeomorphic with respect to $[M_i]$, then the sequences $[M_i]$ and $[T_i(M_i)]$ are said to be *mutually uniformly homeomorphic.*

Note that if for every integer i we have $M_i = M_{i+1}$ and $T_i = T_{i+1}$, then the condition that the sequences be mutually uniformly homeomorphic is equivalent to the condition that a uniform homeomorphism exist between the sets M_i and $T_i(M_i)$, as previously defined by the present writer.[2]

3. *Homeomorphisms.*—We shall next give two sets of conditions under which a homeomorphism exists between the sets of common points of two sequences.

THEOREM 1. *In a metric space, let* $[M_i]$ *and* $[N_i]$ *be sequences of point sets such that for each value of* i, M_i *contains* M_{i+1} *and* N_i *contains* N_{i+1}, *and let* M *and* N, *respectively, denote the sets of points common to all the sets of the sequences* $[M_i]$ *and* $[N_i]$. *For each value of* i, *let* T_i *denote a homeomorphism such that* $T_i(M_i) = N_i$. *Let the sequences satisfy the further conditions:*

(1) *if* p *is any point of* M, *the sequence* $[T_i(p)]$ *converges to a point* P *of* N;

(2) *if* Q *is any point of* N, *the sequence* $[T_i^{-1}(Q)]$ *converges to a point* q *of* M;

(3) *the sequences* $[M_i]$ *and* $[N_i]$ *are mutually uniformly homeomorphic.*

Under these conditions there exists a uniform homeomorphism T *such that* $T(M) = N$.

Proof.—Let p be a given point of M. Then by (1) and (2), the sequence $[T_i(p)]$ converges to a point P of N and the sequence $[T_i^{-1}(P)]$ converges to a point p' of M. We shall show that $p' = p$.

Let ϵ denote an arbitary positive number, and let δ and k be positive numbers whose existence follows from (3), which are such that if $i > k$

and the distance between the points $T_i(p)$ and P of N_i is less than δ, then the distance between the points $T_i^{-1}T_i(p) = p$ and $T_i^{-1}(P)$ is less than ϵ. Since the sequence $[T_i(p)]$ converges to P, there are an infinite number of points of this sequence for which $i > k$ and whose distance from P is less than δ. Hence there are an infinite number of points of the sequence $T_i^{-1}(P)$ whose distance from the point p is less than the arbitrary number ϵ. It follows that the point p' to which the sequence $[T_i^{-1}(P)]$ converges is the point p.

Thus we have shown that if p is a point of M and if $[T_i(p)]$ converges to the point P of N, then the sequence $[T_i^{-1}(P)]$ converges to the point p. Similarly, if Q is a point of N, and if $[T_i^{-1}(Q)]$ converges to the point q of M, then $[T_i(q)]$ converges to Q. Hence it follows that if p and q are distinct points of M, the points P and Q are distinct, and conversely. We can now define a $(1 - 1)$ reciprocal correspondence T between the points of M and the points of N, by making each point p of M correspond to the point of N to which the sequence $[T_i(p)]$ converges.

It remains to show that T is continuous. By (3) it follows that if ϵ is any arbitrary positive number, there exist positive numbers δ and k such that if $i > k$ and the distance between the points p and q of M is less than δ, then the distance between the points $T_i(p)$ and $T_i(q)$ is less than ϵ. But in that case the distance between the points P and Q to which the sequences $[T_i(p)]$ and $[T_i(q)]$ converge is not greater than ϵ. From this it follows that if the point p is a limit point of any subset of M, the point $T(p) = P$ is a limit point of the corresponding subset of N. Hence limit points are preserved under the transformation T and a similar argument shows that they are preserved under T^{-1}. Hence T is a homeomorphism.

The method of proof shows incidentally that T is uniformly continuous.[2] Hence T is a uniformly homeomorphic correspondence or a uniform homeomorphism.

THEOREM 2. *In a metric space, let $[M_i]$ and $[N_i]$ be sequences of compact point sets such that for each value of i, M_i contains M_{i+1} and N_i contains N_{i+1}. Let M and N, respectively, denote the sets of points common to all the sets of the sequences $[M_i]$ and $[N_i]$. For each value of i, let T_i denote a homeomorphism such that $T_i(M_i) = N_i$. Let the sequence satisfy the further conditions:*

(1) *if p is any point of M, the sequence $[T_i(p)]$ converges to a point P of N;*

(2) *if Q is any point of N, the set M contains every limit point of the set $\sum T_i^{-1}(Q)$;*

(3) *the sequences $[M_i]$ and $[N_i]$ are mutually uniformly homeomorphic.*

Under these conditions there exists a uniform homeomorphism T such that $T(M) = N$.

Proof.—Let Q be a point of N. Then since M_i is compact, the sequence $[T_i^{-1}(Q)]$ has limit points which are points of M, by (2). Suppose it has two limit points x and y. From (1) and (3), it follows that if ϵ is an arbitrary positive number, there exist positive numbers δ and k such that (*a*) the distance between the points $T_i(x)$ and X of N_i is less than ϵ for every $i > k$, (*b*) the distance between the points $T_i(y)$ and Y of N_i is less than ϵ for every $i > k$, (*c*) the distance between the points x and $T_j^{-1}(Q)$ of M_j is less than δ for some $j > k$, and hence the distance between the points $T_j(x)$ and Q of N_j is less than ϵ for some $j > k$, and (*d*) the distance between the points y and $T_h^{-1}(Q)$ of M_h is less than δ for some $h > k$, and hence the distance between the points $T_h(y)$ and Q of N_h is less than ϵ for some $h > k$. From (*a*) and (*c*) it follows that the distance between the points X and $T_j(x)$ of N_j is less than ϵ, and the distance between the points $T_j(x)$ and Q of N_j is less than ϵ, and hence the distance between the points X and Q of N is less than 2ϵ. Similarly from (*b*) and (*d*) it follows that the distance between the points Y and Q of N is less than 2ϵ. Therefore the distance between the points X and Y of N is less than 4ϵ, and since ϵ is arbitrary, it follows that $X = Y$.

But if $X = Y$, it follows from (*a*) and (*b*) that the distance between the points $T_i(x)$ and $T_i(y)$ of N_i is less than 2ϵ for every $i > k$. But then it is easily established by means of hypothesis (3) that the distance between the points x and y of M is less than α, where α is arbitrary. Hence $x = y$. In other words the sequence $[T_i^{-1}(Q)]$ has a single limit point to which it converges. Hence the hypotheses of Theorem 1 are fulfilled and the conclusion follows from Theorem 1.

4. *Homeomorphic Transformations.*—In this section it is shown that if the hypotheses of Theorems 1 and 2 are slightly weakened, we can no longer prove that M and N are homeomorphic, but can merely prove that there exists a continuous $(1 - 1)$ transformation of M into N, concerning whose inverse we know nothing except that it is $(1 - 1)$. We shall call such a transformation a *homeomorphic transformation.*

THEOREM 3. *In a metric space, let* $[M_i]$ *and* $[N_i]$ *be sequences of point sets such that for each value of* i, M_i *contains* M_{i+1} *and* N_i *contains* N_{i+1}, *and let* M *and* N, *respectively, denote the sets of points common to all the sets of the sequences* $[M_i]$ *and* $[N_i]$. *For each value of* i, *let* T_i *denote a homeomorphism such that* $T_i(M_i) = N_i$. *Let the sequences satisfy the further conditions:*

(1) *if* p *is any point of* M, *the sequence* $[T_i(p)]$ *converges to a point* P *of* N, *and if the points* p *and* p' *of* M *are distinct, than the points* P *and* P' *are distinct;*

(2) *if* Q *is any point of* N, *the sequence* $[T_i^{-1}(Q)]$ *converges to a point* q *of* M;

(3) *the sequence* $[N_i]$ *is uniformly homeomorphic with respect to the sequence* $[M_i]$.

Under these conditions there exists a homeomorphic transformation t, such that $t(M) = N$.

Proof.—As in the proof of Theorem 1, we can show that if P is a point of N and if the sequence $[T_i^{-1}(P)]$ converges to the point p of M, then the sequence $[T_i(p)]$ converges to P.

Suppose that there is a point p of M such that $[T_i(p)]$ converges to the point P of N, but that $[T_i^{-1}(P)]$ converges to a point q of M, where $q \neq p$. From the preceding paragraph we see that since $[T_i^{-1}(P)]$ converges to q, the sequence $[T_i(q)]$ converges to P. But then we have both $[T_i(p)]$ and $[T_i(q)]$ converging to P, which is contrary to (1). Hence if p is any point of M and if $[T_i(p)]$ converges to the point P of N, then $[T_i^{-1}(P)]$ converges to p.

Thus we define a $(1 - 1)$ correspondence t between the points of M and the points of N by making each point p of M correspond to the point of N to which the sequence $T_i(p)$ converges. As in the proof of Theorem 1, we can show that if p is a limit point of any subset of M, then the corresponding point of N is a limit point of the corresponding subset of N. Hence we have defined a homeomorphic transformation t such that $t(M) = N$.

THEOREM 4. *In a metric space, let $[M_i]$ and $[N_i]$ be sequences of compact point sets such that for each value of i, M_i contains M_{i+1} and N_i contains N_{i+1}. Let M and N, respectively, denote the sets of points common to all the sets of the sequences $[M_i]$ and $[N_i]$. For each value of i, let T_i denote a homeomorphism such that $T_i(M_i) = N_i$. Let the sequences satisfy the further conditions:*

(1) *if p is any point of M, the sequence $[T_i(p)]$ converges to a point P of N, and if the points p and p' of M are distinct, then the points P and P' are distinct;*

(2) *if Q is any point of N, the set M contains every limit point of the set $\sum T_i^{-1}(Q)$;*

(3) *the sequence $[N_i]$ is uniformly homeomorphic with respect to the sequence $[M_i]$.*

Under these conditions there exists a homeomorphic transformation t, such that $t(M) = N$.

Proof.—As in the proof of Theorem 2, we show that if Q is any point of N, and the sequence $[T_i^{-1}(Q)]$ has two limit points x and y, the sequences $[T_i(x)]$ and $[T_i(y)]$ converge to the same point of N. But this is impossible by (1). Hence the sequence $T_i^{-1}(Q)$ converges to a single point of M. But in that case the hypotheses of Theorem 3 are satisfied, and the conclusion follows from that theorem.[3]

* Presented to the American Mathematical Society, October 31, 1931.

[1] B. Knaster, *Funda. Math.*, **3**, 247–286 (1921).

[2] H. M. Gehman, *Trans. Amer. Math. Soc.*, **29**, 553–568 (1927).

[3] In a recent paper by J. H. Roberts, *Trans. Amer. Math. Soc.*, **34**, 252–262 (1932), is given a theorem (Theorem 1) closely related to the theorems of this paper. While most of Roberts' results will hold true in a more general space than the one considered by him, the statement that "if π^{-1} is single-valued, it is continuous," will not be true in a general metric space.

AN IMPROVED EQUAL-FREQUENCY MAP OF THE NORMAL CORRELATION SURFACE, USING CIRCLES INSTEAD OF ELLIPSES

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In studying the correlation between two variables X and Y it is often important to compare a given scatter-diagram having observed values of $\overline{X}$, $\overline{Y}$, σ, τ, r, with a normal distribution having the same values of $\overline{X}$, $\overline{Y}$, σ, τ, r. (Here σ and τ denote the standard deviations of the X's and the Y's, respectively, and r denotes the coefficient of correlation.)

The usual method of making such a comparison is to draw the "50 per cent ellipse" on the diagram, and see whether 50 per cent of the dots lie within the ellipse. But this process is rather laborious, and becomes more so if one attempts to plot the "10% ellipse," the "20% ellipse," etc

The purpose of this paper is to show how this complicated family of ellipses can be replaced by a simple family of easily plotted concentric circles. These concentric circles, together with a family of equally spaced radial lines, will form a "cobweb map" which divides the plane into "townships of equal frequency" of any desired fineness of mesh (for example, percentiles, permilles, etc.).

This cobweb map makes possible a direct comparison between the observed distribution of dots and the theoretical distribution in the corresponding normal case.

The process of constructing the map is extremely simple, as follows.

Let figure 1 represent the given scatter-diagram, in which h is the class-interval for X, and k the class-interval for Y, and suppose that the means $\overline{X}$, $\overline{Y}$ (in the original units), the standard deviations σ, τ (in the original units), and the coefficient of correlation r (a pure number), have been computed in the usual way. Now re-draw the diagram in oblique form, as follows (see Fig. 2).

First, rotate the axis of Y through an angle ψ given by $\tan\psi = \dfrac{r}{\sqrt{1-r^2}}$.

Second, choose as the geometric unit of length to be used on the new

diagram a length equal to about $\sqrt{1-r^2} \times 1/8$ (the width of the paper), and denote it by $\pounds(u)$. (For example, we may have $\pounds(u) = 1$ inch, or 1 cm. on the diagram.[1]) Compute the geometric lengths $\pounds(h) = \frac{h}{\sigma} \frac{1}{\sqrt{1-r^2}} \pounds(u)$ and $\pounds(k) = \frac{k}{\sigma} \frac{1}{\sqrt{1-r^2}} \pounds(u)$, to represent on the diagram the class-intervals h and k. Then, laying off successive multiples of $\pounds(h)$ and $\pounds(k)$ along the new axes of X and Y, re-draw the given checkerboard in the oblique form indicated in figure 2. In this new diagram we shall have $\pounds(\overline{X}) = (\overline{X}/h)\pounds(h)$ and $\pounds(\overline{Y}) = (\overline{Y}/k)\pounds(k)$; and also, incidentally, $\pounds(\sigma) = \pounds(\tau) = \pounds(u)/\sqrt{1-r^2}$.

Third, to find the circle which shall include any given percentage, p, of the total number of dots (where $p = 10\%, 20\%$, etc.), find the value of the coefficient K given by the formula $K = \sqrt{-2 \log_e (1-p)}$, and compute the geometric length $\pounds(a) = K\pounds(u)$. (A table for K as a function of p is provided.) Then using this geometric length $\pounds(a)$ as radius, and the mean point of figure 2 as center, describe a circle. The circle will

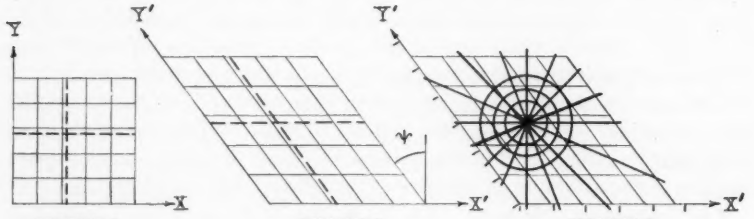

FIGURE 1 FIGURE 2 FIGURE 3

include the given fraction, p, of the total frequency. In this way as many "frequency circles" as desired can be readily constructed.

p	10%	20%	30%	40%	50%	60%	70%	80%	90%
K	0.459	0.668	0.845	1.011	1.177	1.354	1.552	1.794	2.146

Fourth, complete the "cobweb" by drawing as many equally-spaced radial lines (see Fig. 3) as may be desired. (The outermost "townships" extend, of course, to infinity.)

The mapping process here outlined would, it is believed, greatly simplify such applications of statistical theory as Dr. W. A. Shewhart for example has found important in Chapter IX of his recent book on "Economic Control of Quality of Manufactured Products." Also, Professor E. B. Wilson has suggested that the device may be found useful in the study of the random distribution of hits on a target. In general, the process provides a very expeditious graphical method by which the "normality" of any given distribution of two variables may be tested.[2]

[1] The notation $\mathfrak{L}(\sigma)$, which may be read: "*geometric* σ," is introduced in order to keep clear the distinction between a physical magnitude, such as $\sigma = 17$ ounces, and the geometric length which is used to represent this magnitude on the diagram. By the aid of this notation it is possible to adhere strictly to the useful convention that "the equality sign should never be used except between quantities of the same kind." Thus, if $\sigma = 17$ ounces, we may have $\mathfrak{L}(\sigma) = 2.1$ inches on the diagram; but we could not properly write $\sigma = 2.1$ inches, since 2.1 inches is not equal to 17 ounces. [If, however, u is itself a geometric length on the diagram, then $\mathfrak{L}(u) = u$.]

[2] The mathematical proof of the correctness of this construction, together with a more extensive table of the factor K, will be given in a forthcoming paper in the *Journal of the American Statistical Association*, September, 1932.

MODULAR HOMOLOGY CHARACTERS

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This note discusses the theory of modular homology characters founded by Alexander in his paper on *Combinatorial Analysis Situs*[1] (hereafter designated by A. C.), and more recently considered by Lefschetz in his Colloquium Lectures on *Topology*[2] (hereafter designated by L. T.). We preface our work by an elementary treatment of matrices equivalent modulo m (m being any integer > 1 or, suitably interpreted, $= 0$); this treatment is essentially related to the methods used by Alexander in handling modular chains (cf. pp. 319–22 A. C.).

1. *Matrices Equivalent Mod m.*—Let w denote a matrix the elements of which are integers w^c_γ. We say that another such matrix $'w$ is *equivalent mod m* to w if

$$'w^d_\delta \equiv x^d_c \; w^c_\gamma \; y^\gamma_\delta \mod m, \tag{1.1}$$

where x and y are square matrices of integers with determinants $|x|$ and $|y|$ prime to m. This equivalence is clearly reflexive and transitive. It is also symmetric, for

$$w^c_\gamma \equiv {}'x^c_d \; {}'w^d_\delta \; {}'y^\delta_\gamma \mod m, \tag{1.2}$$

where $'x$ is a matrix obtained by taking $'x^c_d = \xi$ times the cofactor of x^d_c in x, ξ being such that $|x| . \xi \equiv 1 \mod m$, and $'y$ is a matrix obtained in similar fashion from y. If $m = 0$ our equivalence mod m becomes ordinary equivalence; we have only to interpret congruence mod 0 as equality and agree that ± 1 — which are the only integral solutions of $|x| . \xi = 1$ — are the only integers prime to 0.

Let $G_r^{(m)}(w)$ denote the G. C. D. of the values of all the polynomials,

$$P_r^{(m)}(w) = a_0 m^r + a_1(w)m^{r-1} + \ldots + a_{r-1}(w)m + a_r(w),$$

which can be formed by taking for a_0 an arbitrary integer and for $a_p(w)$ a form with arbitrary integral coefficients linear in the p-rowed determinants $\Delta_p(w)$ of w. (We agree that the G. C. D. of a set of integers is that of the non-zero members, if any; otherwise it is zero.) The number of $G_r^{(m)}(w)$'s, excluding those (if any) for which $G_r^{(m)}(w) \equiv 0 \bmod m.G_{r-1}^{(m)}(w)$, we call the *rank mod m* of w and denote by $\rho^{(m)}(w)$. Clearly $G_r^{(0)}(w)$ is the G. C. D. of all $\Delta_r(w)$, and $\rho^{(0)}(w)$ the ordinary rank of w.

By expressing (1.1) as

$$'w_\delta^d = \bar{w}_\delta^d + mk_\delta^d, \quad \bar{w}_\delta^d = x_c^d w_\gamma^c y_\delta^\gamma,$$

we have, using familiar properties of determinants, that a $\Delta_p('w)$ is a $P_p^{(m)}(\bar{w})$ and that a $\Delta_q(\bar{w})$ is an $a_q(w)$. Accordingly, a $P_r^{(m)}('w)$ is a $P_r^{(m)}(w)$. In similar fashion it follows from (1.2) that a $P_r^{(m)}(w)$ is a $P_r^{(m)}('w)$. Therefore

$$G_r^{(m)}('w) = G_r^{(m)}(w), \tag{1.3}$$

and so

$$\rho^{(m)}('w) = \rho^{(m)}(w). \tag{1.4}$$

Compatible with our definition of equivalence mod m are the four following *elementary transformations mod m* (cf. the "generating transformations," p. 320 A. C.):

A. The addition of the elements of one row (column) to the corresponding elements of another row (column).

B. The reversal of the signs of all the elements of a row (column).

C. The multiplication of all the elements of a row (column) by one and the same positive integer prime to m.

D. The replacement of any element by a congruent integer mod m.

If $m = 0$, C and D become identity transformations. Clearly the inverses of all four transformations may be achieved by combinations of elementary transformations.

THEOREM. *By elementary transformations mod m the matrix w may be reduced to a unique matrix v consisting of zeros except down the main diagonal where there are elements* $I_s^{(m)}(w)$ $(s = 1, 2, \ldots, \rho^{(m)}(w))$ *such that*

$$I_s^{(m)}(w) = G_s^{(m)}(w)/G_{s-1}^{(m)}(w) \quad (G_0^{(m)}(w) = 1). \tag{1.5}$$

By transformations of types A and B, w can be reduced to the well-known normal form consisting of zeros except for the ordinary invariant factors $I_t^{(0)}(w)$ down the main diagonal. Now using transformations of types C and D, if $m \neq 0$, passage is made to a matrix v in which each $I_t^{(0)}(w)$ is

replaced by the G. C. D. of it and m, or by 0 if it is divisible by m. The number of these non-zero elements is clearly $\rho^{(m)}(v)$, i.e., $\rho^{(m)}(w)$ in virtue of (1.4). We call them the *invariant factors mod m* of w, and denote them by $I_s^{(m)}(w)$. Since by construction each $I_s^{(m)}(w)$ divides its successors, if any, calculation shows that

$$G_s^{(m)}(v) = I_1^{(m)}(w).I_2^{(m)}(w). \ldots . I_s^{(m)}(w),$$

from which (1.5) follows in consequence of (1.3). Taken together (1.3) and (1.5) imply that

$$I_s^{(m)}('w) = I_s^{(m)}(w),$$

thereby showing that the reduced matrix v is unique.

The above theorem has as corollary the fact that the passage from w to any matrix equivalent mod m, say $'w$, can be made by elementary transformations mod m; we have only to reduce w to v by elementary transformations and then reverse those reducing $'w$ to v.

We call the powers of primes occurring in the invariant factors $I_s^{(m)}(w)$ the *elementary divisors mod m* of w. It is readily seen that the elementary divisors mod m of a matrix, whose only elements not $\equiv 0 \bmod m$ lie (anyhow) on a diagonal, are just the highest powers of primes these elements have in common with m.

2. *Homology Characters Mod m.*—Let η^p be the matrix exhibiting the incidences between the $(p+1)$-cells and the p-cells of an n-complex K. Let

$$\rho^{(0)}(\eta^p) = \rho_p, \qquad \rho^{(m)}(\eta^p) = \rho_p';$$
$$I_\lambda^{(0)}(\eta^p) = \zeta_\lambda^p, \qquad I_\mu^{(m)}(\eta^p) = \xi_\mu^p.$$

Let $\{C_p^i\}$ $(i = 1, 2, \ldots, \alpha_p;\ \alpha_p =$ the number of p-cells of $K)$ denote a set of p-chains on K whose coefficients form a unimodular matrix. We can choose[3] a basis $\{C_p^i\}$, for each value of p, such that

$$\left.\begin{aligned} &C_{p+1}^\kappa \longrightarrow 0 \qquad (\kappa = 1, 2, \ldots, \alpha_{p+1} - \rho_p) \\ &C_{p+1}^{\alpha_{p+1}+1-\lambda} \longrightarrow \zeta_\lambda^p C_p^\lambda \quad (\lambda = 1, 2, \ldots, \rho_p) \end{aligned}\right\} \tag{2.1}$$

The group $\Gamma_p^{(m)}$ of p-cycles mod m will be generated by

$$\{C_p^\mu\}, \quad \{C_p^\nu\}, \quad \{C_p'^\sigma\}, \tag{2.2}$$
$$(\mu = 1, 2, \ldots, \rho_p';\ \nu = \rho_p' + 1, \ldots, \alpha_p - \rho_{p-1}';$$
$$\sigma = \alpha_p - \rho_{p-1}' + 1, \ldots, \alpha_p),$$

where

$$C_p'^\sigma = mC_p^\sigma / \xi_{\alpha_p+1-\sigma}^{p-1}.$$

The members of (2.2) are subject to the homologies:

$$\left.\begin{aligned} \xi_\mu^p C_p^\mu &\sim 0 \\ mC_p^\nu &\sim 0 \\ \xi_{\alpha_p+1-\sigma}^{p-1} C_p'^\sigma &\sim 0 \end{aligned}\right\} \bmod m. \tag{2.3}$$

Let $F_p^{(m)}$ denote the sub-group of $\Gamma_p^{(m)}$ generated by the left members of (2.3), and let ω^p denote the diagonal matrix of the coefficients appearing in (2.3). The difference (factor) group $H_p^{(m)} = \Gamma_p^{(m)} - F_p^{(m)}$ is the pth homology group mod m of K.

Let

$$R_p(K, m) = \alpha_p - \rho_p' - \rho_{p-1}', \quad \tilde{R}_p(K, m) = \alpha_p - \rho^{(m)}(\omega^p).$$

These two numbers are both numerical invariants of K; they are candidates for the position of pth Betti number mod m of K. The formula for $R_p(K, m)$ is an obvious generalization of the formula for $R_p(K)$, but on the other hand $\tilde{R}_p(K, m)$ is the maximum number of cycles γ_p mod m between which there holds no relation

$$t_1\gamma_p^1 + t_2\gamma_p^2 + \ldots \sim 0 \mod m,$$

unless all $t \equiv 0 \bmod m$. (If the latter statement were not true the invariant factors of ω^p would not be unique.) $R_p(K, m)$ is the modular Betti number defined on p. 42 L. T., while $\tilde{R}_p(K, m)$ is that implicit in the remarks on p. 35 L. T.[4] The former yields an Euler-Poincaré formula, but not the latter. For π prime

$$R_p(K, \pi^u) = \tilde{R}_p(K, \pi^u). \tag{2.4}$$

This accounts for the single definition of Betti (connectivity) number mod π on p. 319 A. C.

Let $\Theta_h^p(K, m)$ denote the invariant factors $I_i^{(0)}(\omega^p) > 1$. These numerical invariants of K are the torsion coefficients mod m defined on p. 43 L. T. They completely determine the group $H_p^{(m)}$. The reduction of ω^p to normal form is the counterpart of the resolution of $H_p^{(m)}$ into the direct sum of cyclic groups of "nested" orders $\Theta_h^p(K, m)$. On the other hand, let $\tilde{\Theta}_j^p(K, m)$ denote the invariant factors $I_k^{(m)}(\omega^p) > 1$. Since all the diagonal elements of ω^p divide m the $\tilde{\Theta}_j^p(K, m)$ merely differ from $\Theta_h^p(K, m)$ by the loss of $\tilde{R}(K, m)$ m's. Hence $\tilde{R}_p(K, m)$ and the $\tilde{\Theta}_j^p(K, m)$ are numerical invariants characterizing $H_p^{(m)}$.

More important, however, than the characterization of $H_p^{(m)}$ by the invariant factors of ω^p is the characterization by its elementary divisors. This latter corresponds to the resolution of $H_p^{(m)}$ into a direct sum of cyclic groups having orders which are powers of primes.

THEOREM I. *$H_p^{(m)}$ is characterized by the number $R_p(K, m)$ and the elementary divisors mod m of η^p and η^{p-1}.* The proof is immediate, for in consequence of our remark at the end of § 1 we have only to read down

(2.3) to see that the elementary divisors of ω^p are: (1) the elementary divisors mod m of η^p, (2) the powers of primes composing m repeated $R_p(K, m)$ times, and (3) the elementary divisors mod m of η^{p-1}.

Since a passage from the matrices η^p to equivalent matrices mod m does not affect the ranks ρ'_p or the elementary divisors mod m, Theorem I shows that the homology groups $H_p^{(m)}$ are also unchanged. This means that for considerations mod m it is sufficient to know incidences to within a congruence mod m.

Let K have a dual K^*. Then

THEOREM II. *The homology groups $H_p^{(m)}$ and $H_{n-p}^{*(m)}$ of K and K^*, respectively, are isomorphic.*[5] The incidence matrix η^{*n-p-1} of K^* is the same as the transverse (transpose) of η^p except perhaps for a uniform difference of sign. Hence the elementary divisors mod m of η^{*n-p-1} are those of η^p and of η^{*n-p}, those of η^{p-1}. Moreover,

$$R^{n-p}(K^*, m) = \alpha^*_{n-p} - \rho^{*\prime}_{n-p} - \rho^{*\prime}_{n-p-1} = \alpha_p - \rho'_{p-1} - \rho'_p = R_p(K, m).$$

Consequently the proof of II is completed by applying I.

THEOREM III. *The numbers $R_p(K, m)$ for all m which are powers of primes, and for all p, determine all $R_p(K)$ and all $\Theta_g^p(K)$.*[6] Let $m = \pi^u$ and let $t_p^{(u)} = \rho_p - \rho'_p$. Then $t_p^{(u-1)} - t_p^{(u)}$ = the number of times π^u is an elementary divisor of η^p. To calculate each $t_p^{(u)}$ we have the formula

$$R_p(K, \pi^u) - R_p(K) = t_p^{(u)} + t_{p-1}^{(u)}$$

where $t_{-1}^{(u)} = 0 = t_n^{(u)}$ since $\rho_{-1} = 0 = \rho_n$. But $t_p^{(u)} = 0$, all p, for u sufficiently large and so $R_p(K)$ can be calculated. Hence a knowledge of $R_p(K, \pi^u)$, all u and all p, determines $R_p(K)$ and the elementary divisors of η^p which are powers of π, all p. By applying this result to all primes π the theorem is obtained. Due to (2.4) $\tilde{R}_p(K, m)$ might have replaced $R_p(K, m)$ in the statement of the theorem.

[1] J. W. Alexander, *Trans. Am. Math. Soc.*, **28**, 301–329 (1926).

[2] S. Lefschetz, *Topology*, New York (1930). The topological terminology and notation we use are based on this book.

[3] The choice of bases is like that on p. 37 L. T. except that the elements have been permuted so that the reduced matrices have the invariant factors arranged in ascending order up the diagonal originating at the lower left corner.

[4] Professor Alexander in his seminar (April, 1931) discussed the difference between these definitions, and showed that both led to numerical invariants. The matter had been brought to light by an example constructed by Hausdorff and communicated to Alexandroff at Princeton. Čech has recently given an example also.

[5] Cf. L. Pontrjagin, *Math. Annalen*, **105**, 169 (1931).

[6] Cf. pp. 322–323 A. C. The phrase "greater than or equal to π" occurring in the second last line of p. 322, and again in the next sentence, should of course read "divisible by π."

ORDERS FOR WHICH A GIVEN NUMBER OF GROUPS EXIST

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The determination of all the groups which have the same given order has received considerable attention since 1854 when A. Cayley first determined the two possible groups of each of the orders 4 and 6, respectively, but the determination of the possible orders for which a given number of groups exist has received much less attention thus far. In particular, while it is known that there is always at least one group of every given order it is not known that there is at least one order for which the number of existing groups is exactly equal to an arbitrary given number. The present article is devoted to the study of the possible groups and orders when the number of the existing groups is assumed to be some given fixed number.

We shall first consider the case when the order g of the existing groups is not divisible by the square of a prime number. Hence $g = p_1, p_2, \ldots p_\lambda$, where $p_1, p_2, \ldots p_\lambda$ are distinct prime numbers, and it may be assumed without loss of generality that $p_1 > p_2 > \ldots > p_\lambda$. It is well known that such a group is solvable and contains one and only one subgroup of order $p_1 p_2 \ldots p_\alpha$, where $\alpha \leqq \lambda - 1$. From this it results that if the prime numbers $p_1 \ldots p_\lambda$ can be divided into two or more sets such that no number of one of the sets is congruent to unity with respect to some number of another one of these sets then each of the possible groups is the direct product of groups whose orders are the separate products of the numbers contained in these respective sets. In particular, when none of the primes $p_1, p_2, \ldots p_\lambda$ is congruent to unity with respect to another such prime then the cyclic group is the only possible group of order g.

If among the prime numbers $p_1, p_2, \ldots p_\lambda$ it is possible to select a set of k numbers such that each of them except the last is congruent to unity with respect to the following one but not with respect to any other one of these λ factors, while none of the remaining $\lambda - k + 1$ prime factors of g is congruent to unity with respect to one of these factors, then the number of groups of order g is the k^{th} number of the series

$$1, 2, 3, 5, 8, \ldots\ldots$$

where each number after the second is the sum of the two preceding ones. A proof of this theorem results almost directly from the fact that when G is any group whose order g is not divisible by the square of a prime number then if an operator s of order p_α in G transforms an operator t of order p_β into a power of itself which is not congruent to unity with respect to

p_β then s cannot correspond in an automorphism of G to any power of itself unless the index of this power is congruent to unity with respect to p_α.

Suppose that g involves one and only one prime factor p which is congruent to unity with respect to at least one other of its prime factors and that p is thus congruent with respect to k of these prime factors. Hence $p - 1$ is divisible by the product of these k factors. The number of groups of order g is then one more than the sum of the different combinations of these k prime numbers taken one, two, up to k at a time. That is, this number is equal to 2^k. From the theorem noted in the second paragraph it results that it is possible to find, when k is large, in many other ways values of g for which there are exactly 2^k groups. One such way is to determine g as the product of k distinct pairs of prime numbers such that one number of each pair is congruent to unity with respect to the other but that no number of one pair is congruent to unity with respect to a number of another pair.

A necessary and sufficient condition that a group G whose order is not divisible by the square of a prime number is abelian is that it is cyclic and the order of the group of isomorphisms of such a group is a divisor of

$$p_1 p_2 \ldots p_{\lambda-1}(p_1 - 1)(p_2 - 1) \ldots (p_\lambda - 1).$$

When G is the dihedral group whose order is the double of an odd prime number the order of its group of isomorphisms is equal to this product. In all other cases it is less than this product. In the special case when G is cyclic its group of isomorphisms is the direct product of λ cyclic groups whose orders are the last λ of these factors, respectively, and hence its order is equal to the product of these λ factors. Moreover, when its order is equal to this product G must be cyclic.

It is especially easy to determine all the possible groups of a given order g when these groups must be abelian. It is known that necessary and sufficient conditions that all the groups of a given order $g = p_1^{\alpha_1} p_2^{\alpha_2} \ldots p_\lambda^{\alpha_\lambda}$; where $p_1, p_2, \ldots p_\lambda$ are distinct prime numbers are abelian are: (1) Each $\alpha_i \leqq 2$ $(i = 1, 2, \ldots \lambda)$; (2) no $p_i^{\alpha_i} - 1$ is divisible by any of these distinct prime numbers.[1] The number of these distinct groups is then obviously equal to 2^k where k is the number of these exponents which are equal to 2. Hence this gives another method to determine orders for each of which there are exactly 2^k groups, where k is an arbitrary natural number. In particular, there are exactly 8 groups of order $5^2.7^2.17^2$.

When g is the product of distinct prime numbers such that two and only two of these prime factors are congruent to unity with respect to some other such factor and the larger of these is thus congruent with respect to the smaller and to one and only one other number p, which is also the only one of these factors to which the smaller of these two numbers is thus congruent, then the number of the groups of order g is $p + 4$. Hence

there are exactly seven groups of order $3.7.43$. If we assume that the larger of the two given factors as well as the smaller is congruent to unity only with respect to p, while the other conditions remain unchanged, then the number of the groups of order g is exactly $p + 2$. When $p = 2$ and the other two prime numbers in question are 3 and 5, it results from this theorem that the number of possible groups of order 30 is four.[2]

From what precedes it results directly that a necessary and sufficient condition that there are exactly two groups of a given order g is that g satisfies one of the following two conditions: (1) It is not divisible by the square of a prime number and one and only one of its prime factors is congruent to unity with respect to one and only one other such factor; (2) Its order is divisible by the square of one and only one prime number p but by no higher power of p and none of the prime factors of g is congruent to unity with respect to another such factor nor is $p + 2$ thus congruent. Similarly, it results therefrom that a necessary and sufficient condition that there are exactly three groups of a given order g is that g satisfies one of the following two conditions: (1) It is not divisible by the square of a prime number and each of two and only two of its prime factors is congruent to unity with respect to another such factor and these are thus congruent with respect to one and only one other such factor and the larger is thus congruent with respect to the smaller; (2) Its order is divisible by the square of one and only one prime number p but by no higher power of p and none of the prime factors of g is congruent to unity with respect to another such factor but $p + 2$ is thus congruent with respect to one and only one of the prime factors of g. While there are 25 orders less than 100 for each of which there are exactly two groups, 75 is the only such order for which there are exactly three groups. All the orders for which there are exactly three groups are odd.

If there are exactly four groups of order g then g cannot be divisible by the cube of a prime number since there are five groups of order p^3, where p is any prime number. Moreover, g cannot be divisible by the square of more than two of its prime factors and when it is divisible by two such squares G must be abelian and a necessary and sufficient condition which the prime factors of g must satisfy in this case was noted above. In particular, the orders of all such groups must be odd. When g is divisible by the square of one and only one prime number p, then p is not congruent to unity with respect to a prime factor of g, but g may involve one and only one prime factor which is congruent to unity with respect to p but not with respect to p^2 while none of the other prime factors of g is congruent to unity with respect to another prime factor of g nor is $p + 2$ thus congruent. The lowest order for which there are exactly four groups according to this theorem is 28.

There are also exactly four groups of order g in case g is divisible by the

square of only one of its prime factors when one and only one prime factor of g is congruent to unity with respect to one and only one other such factor and the latter is different from p, and none of the prime factors of g divides $p + 1$. When g is not divisible by the square of a prime number and there are exactly four groups of order g, then g involves either one and only one prime factor which is congruent to unity with respect to another of its prime factors and this is thus congruent to exactly two such factors, or it involves two and only two prime factors such that each of them is congruent to unity with respect to one and only one other such factor but not with respect to the same other factor while none of its factors besides the two given ones is congruent to unity with respect to such a factor, and the larger of these two is not congruent to unity with respect to the smaller. Hence there are eight and only eight numbers less than 100 each of which is the order of exactly four groups. These numbers are as follows: 28, 30, 44, 63, 66, 70, 76 and 92.

[1] Miller, Blickfeldt, Dickson, *Finite Groups*, 117 (1916).
[2] O. Hölder, *Math. Annalen*, **43**, 412 (1893).

GEOMETRY OF THE HEAT EQUATION: FIRST PAPER

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1. In this and some subsequent papers we wish to consider an important physical type of family of plane curves, for which we propose the name *heat family* or *family of heat curves*.[1]

A set of heat curves may be defined as follows. Suppose that heat is flowing by conduction in any way in the plane. At any fixed time draw all the curves of constant temperature; these are the ∞^1 *isothermals* at that time. As the time varies, the isothermals will, in general, change. The complete set of ∞^2 isothermals for all instants of time is what we shall understand by a family of *heat curves*.[2] Heat curves are therefore the loci of constant temperature throughout a given flow of heat. In general, they form a two-parameter family, the parameters being temperature and time; but in certain cases to be discussed later, the family may degenerate into merely ∞^1 curves.

Heat families should not be confused with the ordinary isothermal families defined by the Laplace equation. These are the ∞^1 isothermals defined by a steady flow of heat, and thus constitute a very special case of

a degenerate heat family. This special case, of course, occurs frequently in physics, geometry and function theory. The general case apparently has not been studied hitherto, and we wish to study it here especially from the geometric aspect.

The mathematical formulation is simple. The temperature v is a function of position x, y and time t, say, $\varphi(x, y, t)$. The equation

$$v = \varphi(x, y, t) \tag{1}$$

defines the set of heat curves, if we think of v and t as parameters. The function $\varphi(x, y, t)$ here is any solution of Fourier's heat equation (for planar flow),

$$\frac{\partial^2\varphi}{\partial x^2} + \frac{\partial^2\varphi}{\partial y^2} = k^2 \frac{\partial\varphi}{\partial t} \tag{2}$$

where k is a physical constant depending on the conductivity, density and specific heat of the (homogeneous, isotropic) plane. For this reason we speak of our problem as *the geometry of the heat equation.*

2. If a double infinitude of curves is given arbitrarily it will not in general be possible to identify it with a heat family. Such a family must therefore have characteristic geometric properties which are apparently very complicated and will be discussed later. In the present paper we raise and answer two questions of this nature: when does it happen that all of a set of heat curves are of a special geometrically simple type? In particular, when are all the curves straight lines? When are all of them circles? The questions are interesting in themselves, and for later, more general work it is important to have the answers. Our results are:

THEOREM I: *The ∞^2 straight lines of the plane do not make up a family of heat curves.*

THEOREM II: *No ∞^2 circles of the plane can form a family of heat curves.*

However, it is possible for the two explicit parameters in (1) to reduce to only one essential parameter. When this happens, we speak of the ∞^1 curves (1) as a *degenerate* family of heat curves. Degenerate families of straight lines and circles do occur, as follows.

THEOREM III: *If all of a family of heat curves are straight lines, the family is degenerate, and is of one of two kinds:*

(*a*) *A set of parallel lines.* The flow need not be steady. If it is, the temperature varies linearly. See equations (15), (16) below.

(*b*) *A pencil of lines.* The flow is steady. The angle of the pencil is linear in the temperature.

THEOREM IV: *If all of a family of heat curves are circles, the family is degenerate, and is of one of three kinds:*

(*a*) *A set of concentric circles.* The flow need not be steady. If it is,

the radius is exponential in the temperature. See equations (26), (27) below.

(*b*) *A pencil of circles through two fixed points.* The flow is steady. The angle of the pencil (the angle between the circles) is linear in the temperature.

(*c*) *A pencil of circles tangent to a fixed line at a fixed point.* The flow is steady. The reciprocal of the radius is linear in the temperature.

In short, *only pencils of straight lines or circles can occur as heat families.*

3. For the proofs we first derive some *equivalent forms of the general heat equation* (2), which we shall need also in other discussions.

By changing the unit of time, if necessary, we can assume that the constant k^2 in (2) is numerically equal to unity. Using the subscript notation for derivatives, and Δ for the Laplacian operator, we write (2) as $\Delta\varphi = \varphi_t$, that is,

$$\varphi_{xx} + \varphi_{yy} = \varphi_t. \tag{3}$$

The family (1) may be given in the general implicit form

$$F(x, y, \nu, t) = 0. \tag{4}$$

This is an identity if we replace ν by $\varphi(x, y, t)$. Differentiating with respect to x, y and t, we find expressions for the derivatives of φ in terms of the derivatives of F. Substituting them in (3), we find

$$F_\nu^2(F_{xx} + F_{yy} - F_t) - 2F_\nu(F_xF_{x\nu} + F_yF_{y\nu}) + F_{\nu\nu}(F_x^2 + F_y^2) = 0. \tag{5}$$

Equations (4) and (5) may be taken to characterize heat curves instead of (1) and (3). Of course, $F_\nu \not\equiv 0$.

Equation (1) may also be given in the form

$$y = f(x, \nu, t). \tag{6}$$

This is the special case of (4) where $F \equiv y - f$. The heat equation for (6) is therefore

$$f_\nu^2(f_{xx} - f_t) - 2f_\nu f_x f_{x\nu} + f_{\nu\nu}(f_x^2 + 1) = 0. \tag{7}$$

If we introduce in place of x, y *minimal coördinates* u, v, defined by

$$u = x + iy, \qquad v = x - iy, \tag{8}$$

equation (1) becomes

$$\nu = \psi(u, v, t). \tag{9}$$

The heat equation (3) (if we change the unit of time appropriately) reduces simply to

$$\psi_{uv} = \psi_t. \tag{10}$$

If (9) is given implicitly by

$$G(u, v, v, t) = 0, \tag{11}$$

the corresponding form of the heat equation is

$$G_v^2 G_t + G_v(G_u G_{vv} + G_v G_{vu} - G_v G_{uv}) - G_{vv} G_u G_v = 0. \tag{12}$$

4. *Discussion for Straight Lines.*—If all of a set of heat curves are straight lines, their equation can be written

$$y = x.\alpha(v, t) + \beta(v, t). \tag{13}$$

This is of the type (6). Hence α and β must be such that (7) is an identity in x, v and t. Substituting, we find that (7) becomes a cubic polynomial in x. The four coefficients therefore vanish identically in v and t; that is, α and β satisfy

$$\alpha_v^2 \alpha_t = 0, \tag{14_1}$$

$$\alpha_v^2 \beta_t + 2\alpha_v \alpha_t \beta_v = 0, \tag{14_2}$$

$$2\alpha\alpha_v^2 - (1 + \alpha^2)\alpha_{vv} + \alpha_t \beta_v^2 + 2\alpha_v \beta_v \beta_t = 0, \tag{14_3}$$

$$2\alpha\alpha_v \beta_v - (1 + \alpha^2)\beta_{vv} + \beta_v^2 \beta_t = 0. \tag{14_4}$$

From (14_1), either $\alpha_v = 0$, or $\alpha_t = 0$.

If $\alpha_v = 0$, $\beta_v \neq 0$, since v must actually be present in (13). Then from (14_3), $\alpha_t = 0$. Thus α is constant, and (13) is a family of parallel lines. We may assume that the lines are parallel to the x-axis; that is, that $\alpha = 0$. Equation (13) is then

$$y = \beta(v, t), \tag{15}$$

and from (14_4), β is any solution of

$$\beta_v^2 \beta_t - \beta_{vv} = 0. \tag{16}$$

This is the case of rectilinear flow. Equation (16) is essentially the heat equation in one dimension, namely,

$$v_{yy} - v_t = 0. \tag{16'}$$

If $\alpha_v \neq 0$, $\alpha_t = 0$. From (14_2), $\beta_t = 0$. Hence the flow is steady, so that the family (13) is again degenerate. At this point Theorem I is therefore proved.

Since $\alpha_t = \beta_t = 0$, α and β are determined from (14_3) and (14_4). These reduce to

$$\frac{\beta_{vv}}{\beta_v} = \frac{\alpha_{vv}}{\alpha_v} = \frac{2\alpha\alpha_v}{1 + \alpha^2}. \tag{17}$$

Thus β is linear in α. This means that the lines (13) form a pencil. We

may assume that the vertex of the pencil is at the origin, so that $\beta = 0$. The solution of (17) is

$$\alpha = \tan (a\nu + b), \tag{18}$$

and (13) becomes

$$y = x \tan (a\nu + b). \tag{19}$$

That is, the angle of the pencil is linear in ν. This completes the proof of Theorem III.

5. *Discussion for Circles.*—If all of a set of heat curves are circles, their equation can be written in the minimal coördinates (8) as

$$[u + \beta(\nu, t)]\,[v + \alpha(\nu, t)] = \rho(\nu, t). \tag{20}$$

In x, y coördinates this is

$$\left[x + \frac{1}{2}(\alpha + \beta)\right]^2 + \left[y + \frac{i}{2}(\alpha - \beta)\right]^2 = \rho. \tag{21}$$

We employ the abbreviations

$$U = u + \beta, \qquad V = v + \alpha. \tag{22}$$

Equation (20) is of the type (11). Hence (12) must be satisfied. This gives

$$(\alpha_\nu U + \beta_\nu V - \rho_\nu)^2 (\alpha_t U + \beta_t V - \rho_t) + \rho_\nu(\alpha_\nu U + \beta_\nu V - \rho_\nu) - \rho(\alpha_{\nu\nu} U + \beta_{\nu\nu} V + 2\alpha_\nu\beta_\nu - \rho_{\nu\nu}) = 0. \tag{23}$$

Here α, β and ρ must be such that this is an identity in u, v, ν, t (and therefore in U, V, ν, t), in consequence of the relation (20), $UV = \rho$. If we set in turn $U = \rho/V$ and $V = \rho/U$ in (23), we can read off from the leading coefficients in the two cases

$$\beta_\nu^2\beta_t = 0, \qquad \alpha_\nu^2\alpha_t = 0. \tag{24}$$

There seem to be four cases here, namely: $\alpha_\nu = \beta_\nu = 0$, $\alpha_t = \beta_t = 0$, $\alpha_t = \beta_\nu = 0$ and $\alpha_\nu = \beta_t = 0$. However, it is easy to see that the last two reduce to the first two, since the coördinates of the center in (21) are to be real. (Non-real circular solutions have been discussed by G. Comenetz in my seminar.)

If $\alpha_\nu = \beta_\nu = 0$, (23) is

$$\rho_\nu^2(\alpha_t U + \beta_t V - \rho_t) - \rho_\nu^2 + \rho\rho_{\nu\nu} = 0. \tag{25}$$

Again using $UV = \rho$, we find that $\alpha_t = \beta_t = 0$. (21) is therefore a set of concentric circles, whose center we may assume to be the origin. The equation is

$$x^2 + y^2 = \rho(\nu, t), \tag{26}$$

where ρ is any solution of

$$\rho_\nu^2(1 + \rho_t) - \rho\rho_{\nu\nu} = 0. \tag{27}$$

If the flow is steady, $\rho_t = 0$, and ρ is found to be the exponential of a linear function of ν. This proves part (a) of Theorem IV.

If $\alpha_\nu \neq 0$ and $\beta_\nu \neq 0$, then $\alpha_t = \beta_t = 0$. Now employing $UV = \rho$ on (23) again, we see that $\rho_t = 0$. Hence the flow is steady, so that the family (21) is again degenerate. At this point Theorem II is therefore proved.

Substituting $\alpha_t = \beta_t = \rho_t = 0$ in (23) and applying $UV = \rho$ finally to the remainder, we find

$$\rho_\nu\alpha_\nu - \rho\alpha_{\nu\nu} = 0; \quad \rho_\nu\beta_\nu - \rho\beta_{\nu\nu} = 0; \quad \rho_\nu^2 - \rho\rho_{\nu\nu} + 2\rho\alpha_\nu\beta_\nu = 0. \tag{28}$$

From the first two of these relations, α is linear in β. This means that the circles (21) have their centers on a line. We may take the line to be the x-axis; then $\alpha = \beta$. The equations (28) become

$$\rho_\nu\alpha_\nu - \rho\alpha_{\nu\nu} = 0, \qquad \rho_\nu(\alpha^2 - \rho)_\nu - \rho(\alpha^2 - \rho)_{\nu\nu} = 0. \tag{29}$$

Integrating,

$$\alpha_\nu = c\rho, \qquad \rho = (\alpha - b)^2 + a. \tag{30}$$

The second equation shows that the circles pass through a fixed point $(b, \sqrt{a})$. Taking the point on the y-axis, $b = 0$. (30) then furnishes two solutions for α. The corresponding forms of (21) are (changing the temperature scale)

$$(x - \sqrt{a}\tan\nu)^2 + y^2 = a\sec^2\nu, \tag{31}$$

$$\left(x - \frac{1}{\nu}\right)^2 + y^2 = \frac{1}{\nu^2}. \tag{32}$$

Equation (31) represents a pencil of circles through two distinct (real or imaginary) points, and (32) is a pencil tangent to a fixed line at a fixed point. Moreover, in (31) the angle between the circles is linear in ν, and in (32) the radius is $1/\nu$. This completes the proof of Theorem IV.

In our next papers we shall discuss all possible degenerate heat families and the extension to space (families of surfaces). We find that there are three distinct types, defined by the equations of Laplace $\Delta u = 0$, Poisson (special) $\Delta u = 1$, and Helmholtz-Pöckels $\Delta u = \pm u$.

[1] The results were presented before the National Academy in 1917. See abstracts in *Bull. Amer. Math. Soc.*, **23**, 273, 302 (1917); **24**, 173 (1918).

[2] A simple example of a doubly infinite heat family, given in my Seminar by V. Perlo, is $y + b = e^{x+a}$, where $a = t$ = time, and $b = \nu$ = temperature. Here the curves are all congruent. A generalization for the translation group was worked out by G. Comenetz. The transformations of the heat equation into itself were studied by A. Fialkow.